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INCREASE OF PATHOGENICITY IN TOMATO-WILT FUSARIUM

FREDERICK L. WELLMAN

(Accepted for publication June 26, 1942)

The tomato-growing industry has long experienced serious losses from the wilt caused by *Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R. Many workers are engaged in the development of wilt-resistant tomato varieties and it has become necessary to know more about changes in pathogenicity that may occur in the causal fungus.

The general practical and theoretical considerations of variability in pathogenic fungi have been discussed by many workers and summarized by Stakman (11), Brierley (2), and Das Gupta (4). The more immediate questions and implications of the effect of this variability on the tomato-wilt-resistance breeding program have been indicated by Boswell (1) and Haymaker (5). The studies reported here were designed to add to the essential understanding of the possible variations in virulence that must be considered in connection with testing wilt-resistant tomato stocks.

These studies are concerned with variability in the pathogenicity of the isolates employed in connection with disease resistance. Past experience has shown (15, 16) that under the usual laboratory handling, the virulent types of the organism commonly exhibit an unquestionable tendency to change, through saltant action, to mild types. However, it is well known that a relatively high virulence of the pathogen appears to be maintained over a long period of time in "wilt-sick" fields. This sustained virulence in nature had seemed to be somewhat in disagreement with the laboratory findings of a general tendency of variation towards milder pathogenicity, although there has been some suggestion (16) that the variation is not always "downward." This later investigation has added substantial evidence in support of variation towards higher virulence.

MATERIALS AND METHODS

The purpose of this work was to determine, first, whether an increase of virulence of the wilt fungus occurred through variation in culture; second, the relative hardiness of mild and virulent types to conditions that may select out the latter, and third, comparative competitive capacities of virulent and mild types of the pathogen in culture and in the host.

The media employed were Tochinai's liquid medium (15) and standard potato-dextrose agar (15), and Wellman's differential medium (14). All Fusarium isolates were from the collection used in earlier studies (15, 16). The reselected and "pure-lined," mild type A15–8 and the virulent R5–6, used for comparative and reference purposes, have been studied and described elsewhere (14). The tomato varieties Bonny Best and Marglobe were the same as those employed in earlier work (12, 15, 16). All cultures were incubated at 28° C. Those aged were held at uncontrolled laboratory temperatures without attempting to reduce desiccation.

In spore-germination and single-spore-isolation studies, the suspensions were spread over hardened agar surfaces. Dilutions were such that around 5 to 20 spores occurred in a microscopic field under low power magnification. Around 30 microscopic fields were examined in marked areas in each Petri dish. Germination of conidia was studied after 18 to 32 hours of incubation and that of chlamydospores after 64 to 68 hours. Where spores appeared dead they were incubated 5 days before being discarded. Only well-isolated germinating spores were used for transfer, and these were picked from agar surfaces after growth was macroscopically visible.

Estimates were made of the number of spores included in a germination series as follows: 20 loopfuls of spore suspension from a loop 1 mm. in diameter, were placed on isolated spots on 2 per cent agar. Spores in the drops were then counted under the microscope and an average per loopful was thus obtained. An estimate of the number of spores was made from the number of loopfuls of suspension spread on agar surfaces to be incubated.

Five growth classes of the genus *Fusarium* have been defined elsewhere (14, 15, 16), but, for convenience, these are herewith briefly described as they appeared on Wellman's differential medium. Relative pathogenicities characteristic of the classes (Table 1) also are indicated.

TABLE 1.—*Pathogenicity values of the tomato wilt Fusarium based on disease effects on tomato seedlings at the 8-leaf stage, after inoculation and growth under standard conditions*

Pathogenicity value[a]	Descriptive designation	Occurrence of darkened vascular bundles		Collapse of stem
		In hypocotyl	In epicotyl	
0	Healthy	None	None	None
1	Mild	In part of tap root	"	"
2	"	In all of root	"	"
3	"	Up to cotyledons	"	"
4	Medium	.....	Above cotyledons	"
5	"	.....	Above juvenile leaves	"
6	Severe	.....	Above leaf 3	"
7	"	.....	Above leaf 4	"
8	Very severe	.....	Above leaf 5	"
9	"	.....	Above leaf 7	"
10	Dying	.....	In apical bud	Tip wilted
11	"	.....	.....	Tip drooping
12	"	.....	.....	Upper third collapsing
13	"	.....	.....	Upper half collapsing
14	"	.....	.....	Upper 4/5th collapsing
15	Dead	.....	.....	All collapsed

[a] Compare with values defined in another report (12).

(R) Raised aerial mycelium, fine woolly white; slight reticulations over mat; undermat tender (easily cut), of light color; no appressed halo about colony. Pathogenicity around 15 to 13; most virulent of the "V" types.

(RS) Raised aerial mycelium with sclerotoid bodies; hyphae mostly fine with some coarse strands, white and fluffy; undermat tender, with dark

sectors; edges of colony often appressed. Pathogenicity 15 to 8; very irregular but considered a "V" type.

(IR) Intermediate raised; aerial mycelium somewhat coarse; narrow, partly appressed halo around colony; undermat light-vinaceous[1] shades, moderately tender. Pathogenicity 9 to 5; moderately virulent "V" type.

(IA) Intermediate appressed; scant, greyish, aerial mycelium, coarse, with wide halo of appressed growth; tough, lavender to tawny undermat. Pathogenicity 5 to 3. Moderately mild "M" type.

(A) Appressed, little or no aerial mycelium; slimy surface; dark vinaceous-purple to tawny, with tough mat. Pathogenicity 3 to 0. The mildest of the "M" types.

Bonny Best plants were employed in all pathogenicity tests, and disease values were obtained by the standard technique already described (12). In the present studies, however, values were recorded in all classs from 0 to 15 as indicated (Table 1).

In the plant-inoculation tests, cultures were held until, according to previous findings (14), mats had attained approximately their heaviest growth in the flasks of liquid medium. Cultures of V types were grown 9 days, those of the M types 22 days; when a culture was composed of the 2 types combined and in the same flask, it was held 15 days. Mats were removed from the liquid, drained a few minutes on filter paper, and the quantity of growth determined as the amount of water displaced by a mat. In preparing inoculum, a 10-cc. volume of fungus growth was added to each 190 cc. of water. When V and M isolates were grown separately and then mixed just before use as inoculum, a 5-cc. volume of each mat was included to make the final 200 cc. of suspension. In all cases the fungus material was thoroughly beaten 3 minutes in a Waring Blendor to insure thorough distribution of finely divided particles of the pathogen in the suspension.

INCREASE OF VIRULENCE BY USE OF CULTURE METHODS

Development of Virulent Saltants in Colonies of Mild Type

It has been repeatedly observed that the usual saltations in tomato wilt Fusarium cultures have been from the R type "down" towards A (15, 16), that is from comparatively high virulence (V) towards definite mildness (M) in pathogenicity. Many thousand mass cultures from 62 "parent" isolates were used, all of which were originally of monosporic origin (14, 15). All isolates of the raised or intermediate classes produced appressed sectors in culture.

In the present studies each variant that appeared in each one of the more than 3000 IA and A cultures examined was always compared with the isolate from which it came. The agar plate cultures each had from 1 to 9 distinctly visible sectors differing in appearance from the rest of the mycelium in the plate. In only 8 cases were the sector variants of a distinctly more raised, or virulent (V) type than the definitely appressed, or mild (M) culture from

[1] All color designations used in this paper are according to standards given in Ridgway (9).

which they came. Tests were made of these eight variants, run in parallel with the cultures of their origin and with known typical V and M types of the organism. It appeared (Table 2) that the differences in culture class

TABLE 2.—*Comparative virulence of 8 sector variants of the tomato wilt Fusarium found in two out of a total of about 3000 colonies*[a] *of mildly pathogenic type*

Culture class	Isolate tested	Pathogenicity[b] value, mean and S.E.
A[c]	(Parent culture)	1.70 ± 0.02
IA	Sector a	2.55 ± 0.02
IA	Sector b	3.15 ± 0.23
IA	Sector c	3.50 ± 0.47
IR	Sector d	5.40 ± 1.80
IR	Sector e	5.40 ± 0.55
IR	Sector f	6.45 ± 0.32
IA	(Parent culture)	3.73 ± 0.28
IR	Sector g	5.50 ± 0.58
R	Sector h	14.85 ± 0.01
A	A15–8[d]	1.00 ± 0.00
R	R5–6[d]	14.00 ± 0.34

[a] Each of these colonies except the two mentioned had from 1 to 9 distinct sectors of dark and appressed types differing in appearance from the rest of the Mycelium.
[b] Result of evaluations (cf. table 1) on 20 Bonny Best tomato plants.
[c] For description of culture-class designations see "Materials and Methods" above.
[d] Standard type (14) included for reference purposes.

of the variants that were more highly raised than their "parents" were definitely higher in pathogenicity.

The difference both in appearance and virulence between the light-colored IR sector f and the dark colored, slimy A colony whence it came was significant. The numerical pathogenicity from a test of the dark colored, slimy A "parent" colony was 1.70, but the fluffy IR sector f had a pathogenicity of 6.45. Another especially significant case was that of sector h, which was fully raised with a white woolly growth, but from a dark slate-purple mat of IA class with coarse, scanty mycelium over its surface. This h sector was highly virulent, with a pathogenicity value of 14.85, whereas its "parent" was mild in virulence with a value of 3.73.

Isolates from Survivals of Thermal-Death-Point Tests

Thermal-death-point tests[2] were first instituted to see if there were differences in this respect between the mild (A15–8) and virulent (R5–6) isolates. Four tests (Table 3) were made and monoconidial isolates from survivors of the treatments were secured for observations as to possible growth variation in culture. The death point for conidia of the virulent culture was approximately 60° C., and that of the mild culture, a little above 50° C. Chlamydospores from either culture survived ten minutes' exposure to 60° C. This was probably close to the highest temperature they

[2] Made according to approved technique. Spore dilutions were from month-old potato-dextrose-agar cultures. Dilutions were in thin-walled test tubes, treated for 10-minute periods in a constant-temperature bath equipped with a standardized thermometer.

TABLE 3.—*Combined results of 4 thermal-death-point studies of conidial suspensions of virulent (R5–6) and mild (A15–8) isolates of tomato wilt Fusarium from which monoconidial isolations of survivors were secured to study for increased virulence*

Temperature treatment[a]	Germination of conidia[b]		Number of monoconidial isolates made[c]		Chlamydospore germination	
	Virulent	Mild	Virulent	Mild	Virulent	Mild
Control[d]	2822	2026	32	232	798	342
45°	2841	1216	0[e]	0[e]	.....	.....
50°	2245	504	0[e]	281	.....	.....
55°	232	0	206	.....	187	199
60°	4	0	4	.....	102	13
65°	0	0	.....	.....	0	0
70°	0	0	.....	.....	0	0
75°	0	0	.....	.....	0	0

[a] Treated for 10 minutes, all temperatures °C.
[b] Average number of survivors out of approximately 3000 conidia per test.
[c] On culturing no significant variations were observed in appearance from that characteristic of the culture of the isolate of their origin.
[d] At room temperature, about 24° C.
[e] No isolates made, since this treatment caused comparatively little destruction of conidia.

could withstand, since, when thus treated, their germination was greatly reduced. The largest percentage of chlamydospore survival after heating at 60° C. was recorded for the most virulent culture. None of the rather limited number of monoconidial isolates from survivors of these tests showed any significant deviation in culture appearance. It is, therefore, probable that there was no significant deviation in virulence among these.

Isolates in Aged Cultures

Isolates that varied considerably in appearance and relative virulence were studied for differences in ability to withstand aging. Test-tube cultures on standard potato-dextrose-agar slants were stored in the laboratory for 1 to 40 months. From conidial suspensions the number of spores were plated out to determine and estimate their viability according to a method described under "Materials and Methods." Germination counts were obtained after incubating the spores at 18-, 24-, and 32-hour intervals. Monosporic isolates were made from all tests where germination occurred, and, whenever only a few spores appeared alive from large numbers plated out, every germinating spore was isolated that could be assuredly judged a single individual. These were grown on differential agar and in liquid medium. They were then compared with parent cultures for growth class (Table 4). The variants most obviously different in appearance were tested for relative pathogenicities, in comparison with the parents and other known cultures (Table 5).

It appeared (Table 4) that spores of both mild and virulent types were killed when aged 3 or $3\frac{1}{3}$ years. Laboratory storage for $2\frac{1}{3}$ to $2\frac{1}{2}$ years reduced the viability of both virulent and mild types, but was far more destructive of the mild type. Even $1\frac{1}{2}$ years of storage considerably in-

hibited the mild types, while 2 years of storage reduced germinability of the virulent types, but by a comparatively small amount. A single month of standing in the laboratory seemed to have no appreciable detrimental effect on spore germinability of either virulent or mild types.

Without including the data from any raised culture or results from the 1-month and 3- and $3\frac{1}{2}$-year-old cultures, about 2400 spores were plated out from the surfaces of long-aged cultures of isolates "low" in culture class and mild in pathogenicity (Table 4). Of these spores only 489 germinated,

TABLE 4.—*Comparative germination of conidia from mild and virulent isolates of the tomato wilt Fusarium aged 1 to 40 months, together with culture types obtained from growth of samples of surviving spores*

Isolate type and No.[a]	Months aged[b]	Conidia		Distribution of monosporic isolates of different classes[d]				
		Incubated[c]	Germinated	R	RS	IR	IA	A
V, 5R[d]	1	2500	2396	180	0	0	0	0
M, 15A	1	2800	2469	0	0	0	0	380
M, 10IA	18	1000	110	36	11	11	18	4
M, 3IA	18	1000	61	6	1	1	51	2
M, 1A	18	1000	75	0	0	0	15	43
M, 15A	18	1000	103	0	0	0	1	59
V, 5R	24	1800	1593	67	0	0	0	0
M, 15A	24	2000	80	0	0	0	0	70
V, 5R	28	2100	1768	80	0	0	0	0
M, 15A	28	2200	19	0	0	3	10	6
V, 5R	30	3900	188	188	0	0	0	0
M, 10IA	30	3700	3	2	0	1	0	0
M, 3IA	30	2600	0	0	0	0	0	0
M, 1A	30	7100	38	0	0	1	10	27
M, 15A	30	4700	0	0	0	0	0	0
V, 5R	36	1500	0	0	0	0	0	0
M, 15A	36	1500	0	0	0	0	0	0
V, 5R	40	4200	0	0	0	0	0	0
M, 15A	40	4000	0	0	0	0	0	0

[a] V = virulent, M = mild. The isolates were the same as those used in previous studies under the same designations (14, 15 and 16).

[b] Aged at room temperature without guarding against desiccation.

[c] Numbers estimated.

[d] For definition of classes as indicated by letters, see above in this paper under "Materials and Methods."

377 of which were transferred as unquestioned monosporic isolates and studied in comparison with the growth of hyphae from week-old parent and type cultures of the 5 growth classes. Of these monosporic isolates, 91 appeared "higher" in culture class than the original culture from which they came.

Tables 4 and 5 show that isolates of low, intermediate, and high types came from single spores from cultures that appeared fully appressed throughout years of growth on artificial media. In turn intermediate cultures were studied and from them isolates were obtained that appeared completely free from any appressed or intermediate characters. Growth types also occurred among single spore isolates from intermediate cultures in which the isolates were of the two very highest classes.

Pathogenicity tests were made with some of the isolates that differed most

significantly from the type of their parent cultures. All these tests (Table 5), which were made simultaneously under identical conditions, substantiate the observation (Table 4) on culture class variation in monosporic isolates originating from appressed cultures. The parent isolates in these studies were quite similar to the mild type A15–8, which is low in pathogenicity and appressed in growth character. It is apparent that from such a seemingly

TABLE 5.—*Comparative pathogenicities of cultures of the tomato wilt Fusarium from representative*[a] *monosporic isolates of spores that survived after aging (Compare with table 4)*

Parental culture[b]		Age of culture in months	Monosporic isolate		Pathogenicity[c] mean and S.E.
No. and class	Pathogenic type		Growth class	Pathogenic type	
5R	V	30	R	V	14.00 ± 0.00[d]
		30	R	V	14.60 ± 0.30[d]
15A	M	28	A	M	1.00 ± 0.00
		28	IA	M	2.13 ± 0.01
		28	IR	M to V	3.60 ± 0.02
10IA	M	30	IR	V	4.93 ± 0.31
		18	IR	V	6.67 ± 0.53
		30	R	V	8.73 ± 0.02
		30	R	V	9.20 ± 0.37
		18	R	V	11.60 ± 0.74
		18	R	V	14.45 ± 0.45[d]
3IA	M	18	R	V	8.87 ± 0.54
		18	R	V	11.20 ± 1.20
		18	R	V	12.00 ± 0.68
5R	V	Not aged[e]	R	V	14.00 ± 0.34
15A	M	do[e]	A	M	1.33 ± 0.01[d]
10IA	M	do[e]	IA	M	2.93 ± 0.28
3IA	M	do[e]	IA	M	3.73 ± 0.28
R5–6	V	do[e]	R	V	14.85 ± 0.01[d]
A15–8	M	do[e]	A	M	1.20 ± 0.02

[a] A total of 82 isolates were tested for pathogenicities in a number of series. Those included in this table were all from one series of tests, and were selected to illustrate variation in virulence of monosporic isolates from cultures originally of mild type. Isolates from 5R included for comparative purposes.

[b] See foot note "a" in table 4.

[c] Pathogenicities all determined at the same time, tested on 15 Bonny Best plants except those marked "d."

[d] Data from test on 20 plants.

[e] Included for reference purposes.

weak and so-called "degenerate" type, variation proceeded "upwards" through all the intermediate steps to the highest type of culture class and pathogenicity; this last being comparable to the fully raised and highly pathogenic pure-line selection R5–6.

It is believed that these findings are of special significance. The whole gamut of pathogenicity was represented in these monosporic isolates from old cultures that had been originally of very mild form. Culturally, they ranged from appressed through intermediate forms to fully raised, and, pathogenically, from mild through intermediate to high virulence.

COMPETITIVE GROWTH OF MILD AND VIRULENT ISOLATES

In Agar Cultures

The agar employed was of the differential formula previously devised (14) to exaggerate dissimilar growth characters of A15–8, a typical M isolate, and R5–6, a typical V isolate. The pieces for inoculum all came from just inside the edges of 3-week-old fungus colonies on agar. The pieces were cut in round disks of 3 sizes measuring 5.5 mm. in diameter (large); 3.0 mm. (medium); and 1.0 mm. (small). These disks were always placed with spore-bearing surfaces down on the agar. Combinations of inoculum were arranged in 13 different ways and grown at 28° C. in triplicate, and the test on each arrangement series was run 3 times.

The inoculum arrangements and results from their growth were as follows:

1. Large M disk alone; all new growth was M.
2. Large V disk alone; all new growth was V.
3. Large disks, M on top of V; all new growth was V.
4. Large disks, V on top of M; all new growth was M.
5. Large disks, edge of M placed 25 mm. from V; colonies met about 11 mm. from edge of V.
6. Large disks, M and V placed edge to edge; somewhat more of agar covered with M.
7. Large V disk, with medium M disk on top; all new growth was V.
8. Medium M disk, with large V disk on top; all new growth was V.
9. Large M disk, with medium V disk on top; all new growth was M.
10. Medium V disk, with large M disk on top; all new growth was M.
11. Large M disk with 2 small V disks placed on opposite sides along a diameter; growth mostly M with a comparatively narrow band of V extending in the line of the diameter.
12. Large V disk with 2 small M disks placed on opposite sides along a diameter; growth mostly M with comparatively narrow band of V extending perpendicularly to the diameter.
13. Matched halves of large M and V disks chopped and macerated together for inoculum; growth irregular, predominantly M type, scanty aerial hyphae, center with considerable V growth.

Growth effects from arrangements 5, 6, 11, 12, 13 are illustrated in figure 1.

These studies showed that the M and V isolates were close together in their relative capacities to grow on agar, but the M type was somewhat more aggressive and was able to outgrow the V. This was most strikingly exemplified in arrangements 11 (Fig. 1, D), 12 (Fig. 1, B. C), and 13 (Fig. 1, A), while it is shown to a lesser extent in 5 (Fig. 1, E) and 6 (Fig. 1, F).

In Liquid Cultures

I have already reported (14) irregularities with regard to pH relations in cultures that were composed of V and M isolates growing in combination in

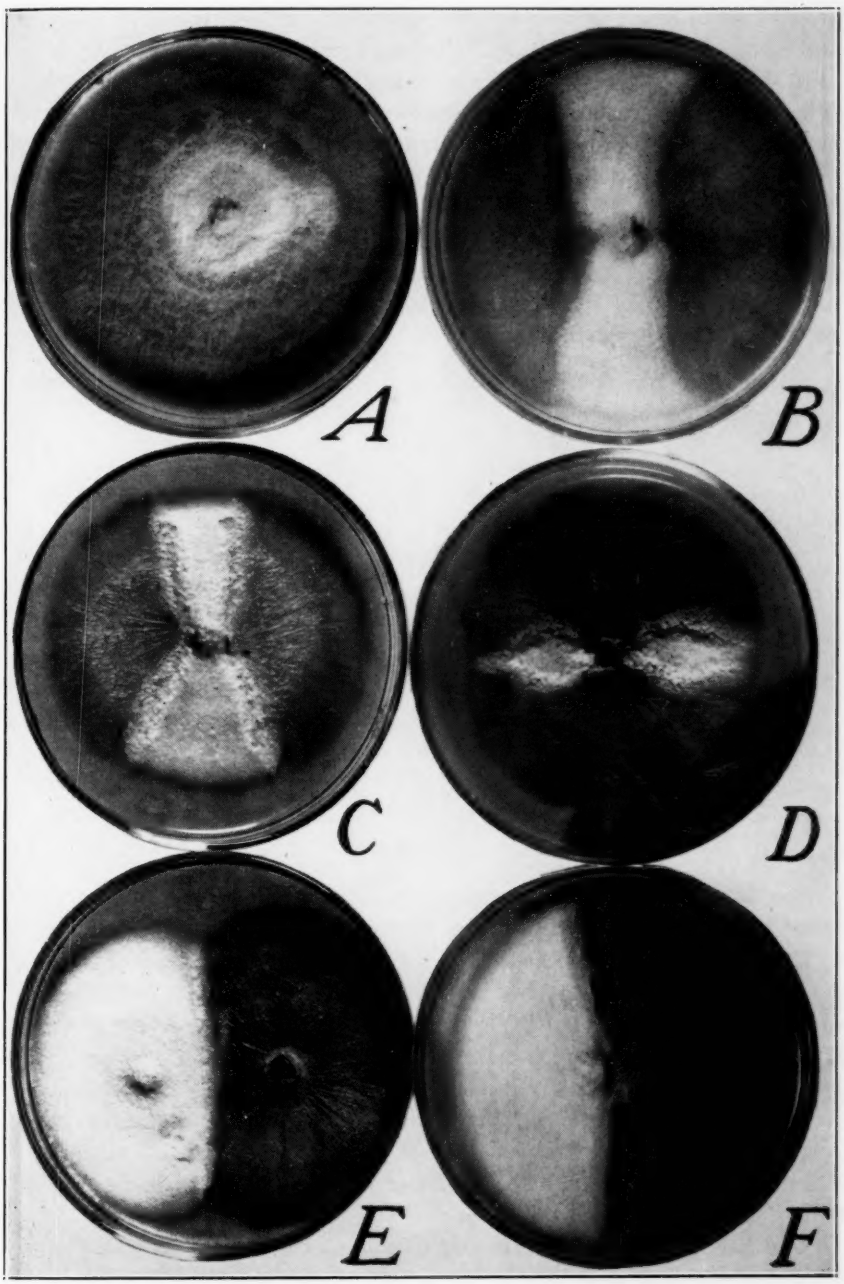

FIG. 1. Growth on agar of the white woolly virulent (V) and the dark slimy mild (M) isolates of *Fusarium bulbigenum* var. *lycopersici*. Inoculum arranged to test competitive growth of the two types. *A*. Matched halves of large, 5.5-mm. disks of V and M chopped and macerated together, planted in center. *B* and *C*. Large disk of V planted with small, 1-mm. disks of M on either side at ends of the transverse diameter. *D*. Large disk of M with small disks of V on either side at ends of the transverse diameter. *E*. Large disks of V and M planted 26 mm. apart. *F*. Large disks of V and M side by side. Note general predominance of mild growth in all arrangements, especially in *A*, *B*, *C*, and *D*.

flasks of liquids. The question of apparent competitive action arose in connection with such studies, and it seemed important to determine in more detail what might result from growth, in liquid, of virulent combined with mild types of the organism. A collection of V and M isolates was studied for changes in pH of the liquids (using techniques already described (14)), and also for appearance of growth on agar after a 14-day incubation period. The results of these studies are to be compared with but are not included in the data presented in table 6. Figure 2 shows the appearance of mats from cultures in a liquid medium of V and M types grown separately as pure cultures and also in combination. It is evident that the V character of growth was the more dominant.

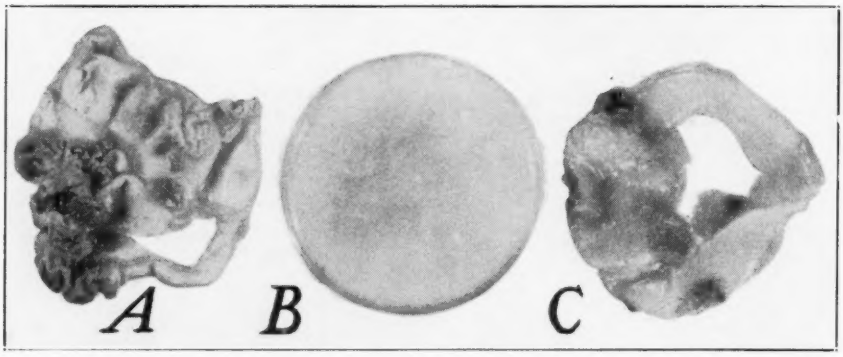

FIG. 2. Under surface of mats of *Fusarium bulbigenum* var. *lycopersici* grown in 100 cc. of Tochinai liquid in flasks. *A*. Pure growth of the virulent type of the organism. *B*. Pure growth of the mild type of the organism. *C*. Combined growth on using inoculum consisting of equal-size disks of both virulent and mild cultures. In liquid cultures the appearance of the combined growth is much nearer to that of the virulent type than to that of the mild.

For 17 pure M isolates all growth on agar was of the typical slimy appressed sort, light tawny to darker, with a few indistinct saltant areas, and a pH range in liquids of from 4.5 to 6.1 with a mean of 5.2. For 26 pure V isolates growth was of the white, woolly, raised sort, practically free from sectors, with a pH range of 7.7 to 8.4 and a mean of 8.1. For the 43 cultures obtained when mixed inoculum from the V and M isolates just described was used in combined cultures in flasks, the growth was variable in character but predominantly of the raised, white, woolly type with many slimy parts of tawny to darker color. In these liquids pH readings ranged from 5.6 to 8.3, with a mean of 7.7. In these studies of combined growth in liquid, the V type of growth appeared predominant.

In other studies of the same sort (Table 6) certain comparative determinations of the mat weights were made, and the competitive action of the V and M types of the *Fusarium* was studied in relation to pathogenicity. These experiments were carried on during two winters. Each experiment is a separate test and the results, therefore, are strictly comparable only within experiments. The data are, however, from experiments that were closely alike as regards the controllable environmental conditions.

TABLE 6.—*Comparative culture character and pathogenicity of virulent (V) and mild (M) isolates of tomato wilt Fusarium when grown in liquid cultures, with pure, combined, and mixed cultures used as inoculum*

Experiment	Isolates	Data[a] on liquid cultures				Pathogenicity[d]	
		Character[b]	Class[c]	Liquid	Mat weight	Mean and S.E.	C.V.
				pH	*mg.*		
1	V–1	Pure	R	8.4	814	13.17 ± 0.31	2.35
	M–1	"	A	5.8	382	0.83 ± 0.01	1.20
	M–2	"	IA	5.9	407	4.75 ± 0.60	12.63
	V–1 + M–1	Combined	R & IR	8.1	593	10.58 ± 0.41	3.88
	V–1 + M–1	"	IR	8.0	512	12.50 ± 0.94	7.52
	V–1 + M–1	"	R	8.3	600	13.00 ± 1.06	8.15
	V–1 + M–2	"	R	8.0	666	8.42 ± 0.40	4.75
	M–2 + M–1	"	A	5.6	412	1.00 ± 0.12	12.00
2	V–2	Pure	R	.....	.....	14.85 ± 0.01	0.07
	M–3	"	A	.....	.....	2.60 ± 0.01	0.38
	V–2 + M–3	Mixed	.....	.....	.....	6.40 ± 0.64	1.00
3	V–3	Pure	R	.....	.....	14.85 ± 0.01	0.07
	M–4	"	IA	.....	.....	2.30 ± 0.02	0.87
	V–3 + M–4	Mixed	.....	.....	.....	6.30 ± 0.57	9.05
4	V–4	Pure	IR	8.0	.....	8.80 ± 0.56	6.36
	M–5	Combined	A	6.2	.....	1.85 ± 0.01	0.54
	V–4 + M–5	"	IR	7.2	.....	8.85 ± 0.47	5.31
	V–4 + M–5	Mixed	.....	.....	.....	3.85 ± 0.63	16.36
5	V–5	Pure	R	8.0	777	13.00 ± 0.33	2.54
	M–6	"	A	5.8	472	1.35 ± 0.01	0.74
	V–5 + M–6	Combined	R	7.6	707	12.07 ± 0.42	3.31
	V–5 + M–6	Mixed	.....	.....	.....	7.10 ± 0.70	9.86
6	V–6	Pure	R	7.9	784	14.10 ± 0.58	4.11
	M–7	"	IA	4.9	464	0.30 ± 0.01	3.33
	V–6 + M–7	Combined	IR & R	7.7	767	13.40 ± 0.66	4.93
	V–6 + M–7	Mixed	.....	.....	.....	7.30 ± 1.63	22.33
7	V–7	Pure	RS	.....	.....	11.40 ± 0.78	6.84
	M–8	"	IA	.....	.....	1.50 ± 0.33	22.00
	V–7 + M–8	Mixed	.....	.....	.....	9.30 ± 1.05	11.29
8	V–8	Pure	R	.....	.....	11.42 ± 0.74	6.48
	V–9	"	RS	.....	.....	11.25 ± 0.81	7.20
	V–8 + V–9	Mixed	.....	.....	.....	11.00 ± 0.87	7.91
9	V–10	Pure	RS	.....	.....	14.45 ± 0.45	3.11
	V–11	"	IR	.....	.....	10.10 ± 0.84	8.32
	M–9	"	IA	.....	.....	5.60 ± 1.53	27.32
	M–10	"	A	.....	.....	0.30 ± 0.01	3.33
	V–10 + V–11	Mixed	.....	.....	.....	13.80 ± 0.73	5.29
	M–9 + M–10	"	.....	.....	.....	2.20 ± 0.51	23.18
10	M–11	Pure	IA	.....	.....	4.75 ± 0.63	12.63
	M–12	"	A	.....	.....	0.83 ± 0.01	1.20
	M–11 + M–12	Mixed	.....	.....	.....	1.00 ± 0.12	12.00

[a] Observations in triplicate.

[b] Using standard techniques (14) and incubated 12 days; pure = pure culture of isolate; combined = culture planted with equal bits of different isolates; mixed = equal portions of isolates that had grown as pure cultures were then mixed and thus used simultaneously as inoculum.

[c] See footnote "d," table 4.

[d] Each value represents a test on 20 plants of Bonny Best variety.
S.E. = standard error; C.V. = coefficient of variation.

Only a few mat weights were obtained, but it appears (Table 6) that when isolates were combined and grown in the same flask, they produced a weight, pH reaction, cultural appearance, and pathogenicity closer to that characteristic of the virulent than of the mild type. Virulent isolates had an average pathogenicity of well over 12, and mild isolates averaged around 2. However, when cultures of these isolates were mixed together mechanically, the resultant inoculum had an average pathogenic value of about 8. In instances when these M and V types were combined in the same flask and the resulting mat used as inoculum, the pathogenicity was below but close to the same as that produced by the pure V culture of the combination.

Pathogenicity tests of V and M isolates (whether pure, combined, or mixed cultures) were made in order to study further which type of isolate was predominant. Bonny Best tomato plants were inoculated (12). Superficial observations were recorded on the comparable state of wilting in plants of these tests at the time the plants were pulled and dissected for disease evaluation. There seemed to be great differences in uniformity of disease severity. Plants infected by the most typical and stable V or M cultures showed a uniform level of wilting, whether severe or moderate, in all the plants. In tests with plants inoculated with mats that had grown from combined V and M cultures, the level of wilting also was fairly even. On the other hand, the irregularity in wilting was quite noticeable in series inoculated with mechanical mixtures of pure cultures of V and M.

The comparative irregularity just mentioned is clearly indicated by the coefficient of variation as given in data from these studies (Table 6, experiments 3, 4, 5, and 6). Certain pure V and M cultures produced data with a small coefficient of variation. When the same V and M cultures were put together and mechanically mixed, the resultant data gave large coefficients. However, if inoculum came from a culture made of isolates that were of combined growth, the pathogenicity data showed a small coefficient. The coefficient of variation of the data from such a combined culture was of about the same magnitude as that obtained from data characteristic of the individual members of the combination culture.

COMPETITIVE GROWTH OF MILD AND VIRULENT ISOLATES IN TOMATO PLANTS

In the Susceptible Variety Bonny Best

In earlier studies (12, 13, 15, 16) reisolations were made from over 2000 plants that had been inoculated with purified, known types of the Fusarium. It was found that in general reisolations the same type of culture as that used for inoculum was recovered from infected plants.

It was thought advisable in the later work to standardize the method of making isolations; hence, only certain portions of the plants were used. Bits of tissue about 2 mm. square were excised from the vascular regions. In choosing the particular vascular strands to be tested for presence of the Fusarium, darkened bundles indicative of infection were selected where they occurred. If none were discolored, those were used that were an extension of

diseased tissues in the plant part below the point where dissections were made, or a bundle was used that led to an epinastic leaf, it being known (13) that such deflected leaves indicate fusarium infection.

The mild type of Fusarium, A15, has been used in many of the writer's studies (14, 15, 16). In standard tests on Bonny Best tomato, it has been found that most plants, inoculated with A15, show no discolored vascular bundles extending above the cotyledonary node. In these studies a total of 684 plants were thus inoculated and only 16 had darkened vascular tracts above the cotyledons. Reisolations were made from each of these 16 plants. In none of the cultures thus obtained was there a significant difference from A15, either in culture class or pathogenicity. In parallel studies, reisolations were made from over 300 plants infected with the virulent type, R5. Plants thus inoculated soon became severely diseased. On this account reisolations were made from plants in the early and late stages of wilt. In no case did the growth character of these reisolations vary from those typical for R5.

Plants were inoculated with mechanical mixtures of cultures of virulent and mild isolates of the wilt pathogen to determine whether there was competition between the virulent and mild types when they invaded plants. Certain isolates were chosen of widely different pathogenicities (Table 6) that were relatively stable in culture, and had markedly different culture appearances. Pairs were combined for inoculum that would be as nearly unmistakable as possible if reisolated from infected tissues. For example, practically colorless appressed M types were mixed with raised V types having a deep pink undermat; or dark purplish appressed M types were mixed with raised V types having white undermats.

The predominance of growth type as it appeared in mixed reisolation cultures from infected tissues was judged by macroscopic examination of what grew out from the diseased host parts. The type of the *Fusarium* covering the largest proportion of the surface of the reisolation plate was considered predominant. During these studies, 80 reisolation plates were observed on which growth type appeared to be about equally M and V, and all of these, in the end, were recorded as predominantly M. These methods were used regardless of the fact that, as was shown above in this paper and has been previously noted (14), the M type tends to grow over agar more rapidly than the V type. It should be pointed out that this method of determining the predominance of growth type may have resulted in unfair advantage in favor of the M type.

The 5 "parts" of the plant out of which the tissue bits were taken for reisolation were as follows: (1) root, tissues from below the transition region and down into the tap root 2 or 3 cm. below the transition region; (2) cotyledonary node, tissues from slightly above, below, and at the attachment of the cotyledons; (3) base, the internodes and leaf traces at the nodes where the juvenile leaves are attached; (4) middle, the recently mature but tender portion comprising the second internode down from the top leaf and apical bud; (5) apex, just below the apical bud.

Results from these studies show (Table 7) that all reisolates from plants inoculated with the pure V cultures were pure V, and all reisolates from

TABLE 7.—*Relative predominance of growth types in Fusarium reisolates from parts of Bonny Best tomato plants infected with virulent (V) and mild (M) types of the fungus that had been grown separately and then mixed for use as inoculum*

Isolates[a] used for inoculum	Diseased plants		Predominance of growth types, expressed as M/V, in cultures from plant parts[b]				
	Number dissected	Range of disease[c]	Root	Cotyledonary node	Base	Middle	Apex
V–R5 + M–A15[d]	138	1 to 15	56/82[e]	36/97	45/90	30/94	10/82
V2 + M1	20	2 to 15	7/13	2/17	5/13	6/11	5/11
V1 + M2	19	5 to 13	6/13	10/9	7/12	7/11	6/11
V1 + M3	24	8 to 14	10/14	11/13	11/13	5/19	5/18
V3 + M3	16	1 to 4	7/9	5/11	5/11	0/8	2/5
V5 + M1	20	8 to 15	5/15	10/10	3/17	9/10	3/11
V9 + M4	18	1 to 15	8/10	8/10	10/8	4/14	3/15
V1 + M4	20	2 to 12	9/11	7/13	4/14	4/11	3/4
V5 + M5	20	1 to 15	2/18	1/19	1/16	0/12	0/11
V6 + M5	20	4 to 15	2/18	3/17	2/18	4/13	1/10
V7 + M12	20	4 to 15	7/13	5/15	5/15	0/12	2/12
V8 + M12	6	11	0/6	0/6	0/6	0/6	0/6
V9 + M8	10	7 to 15	0/10	0/10	0/10	0/9	0/7
V8 + M10	12	4 to 13	1/11	0/12	1/11	1/7	0/5
V10 + M5	14	1 to 13	0/14	0/11	0/12	1/7	0/7
V10 + M4	20	1 to 15	14/6	16/0	14/2	7/2	2/2
V–R5[d]	101	8 to 15	0/101	0/101	0/100	0/78	0/73
M–A15[d]	89	1 to 4	89/0	14/0	6/0	3/0	0/0

[a] Isolates chosen from those listed in table 6 that were widely different in pathogenic effect. Average value for V types was about 13.2, for M types about 1.5 (see table 1 for disease index values).

[b] Detailed description of plant parts and technique of isolation given in the text.

[c] Expressed in range of disease index (cf. table 1) of the plants used for reisolation purposes. These plants were pulled at irregular intervals so as to reisolate from those that differed considerably with regard to severity of disease.

[d] Included for reference purposes; V–R5 and M–A15 are the author's described R5–6 and A15–8 respectively (14).

[e] Reisolates were cultured and classified according to predominance of growth-type of *Fusarium* emerging from the bits of vascular system dissected from diseased plant parts. Expressed as M/V: numerator = number of plant parts from which the predominantly mild (M) type of pathogen developed; denominator = those from which came the predominantly virulent (V) type.

pure M inoculated plants were recovered as pure M. Reisolations from those seedlings inoculated with a mixture of M and V types of the pathogen were made as follows: 397 plants were used for the reisolation purposes, from which attempts were made to secure isolations from a total of 1985 plant parts. Of these parts, 1745 produced cultures of the pathogen, 1264, or 72 per cent, of which were predominantly V; from cotyledonary nodes, 70 per cent were predominantly V; from base parts above the node, 70 per cent were predominantly V; from middle parts, farther up, 76 per cent were predominantly V; and from apex parts, directly below the terminal bud, 84 per cent were predominantly V. When the isolates from tissues from plants infected with mixed V and M cultures were further cultured, it was observed that 92 per cent of all such cultures consisted of a mixture of both

V and M. Since only 8 per cent of all the cultures from plants inoculated with mixed cultures were found to be pure V types, it appears that both M and V were commonly present in those severely diseased seedlings in even the uppermost parts of the plants. It is, therefore, not alone the presence of the fungus in host tissues but its action that causes serious damage.

Results with 30 plants of the above studies (Table 7) deserve detailed discussion, since they represent the repeated observation that growth of M types of the Fusarium extended much farther into the vascular elements of plants infected with both M and V types than when infections resulted from M types alone. Inoculated plants were grown in parallel series under controlled conditions, and tissues were dissected out for reisolation of the pathogen after 9 days.

The first 10 plants had been inoculated with the M type, A15–8, and all appeared healthy before pulling. The roots of all these plants contained the M type of the Fusarium; and in two plants this type was reisolated from tissues slightly below the cotyledonary node. The second 10 plants were inoculated with the V type, R5–6, and were dying. All reisolations from any part of all these plants were the V type. The third group of 10 plants were inoculated with a mixture of these M and V types of the organism. All the plants were diseased, two being mildly wilted. In one of these the M type predominated half way up the stem; in the other the V type predominated, although the M type was recovering along with the V type up to the farthest point of invasion. From two other plants, more severely diseased, the reisolations showed good growth of both V and M with V slightly predominant; but here, again, the M had grown with the V and was recovered from infected vasculars near the apexes of these diseased plants. In two more of the severely wilted plants from this group a combination of M and V types of culture were recovered from the basal half of the plants, although isolates from the apical tissues of one plant were of pure V type. The last 4 plants of this group were collapsing. In the reisolations from them, mixtures of M and V types of the organism came from all tissues, even from the apices.

In the Resistant Variety Marglobe

Both Bonny Best and Marglobe seedlings were used in these studies (Table 8). The isolates for inoculum came from the same lot as those in table 7. Certain of these were reselected and were designated as a. The V types were chosen among those that were raised, white, and produced nothing but non-septate microspores as conidia, while all M cultures were appressed, dark, and bore typically multiseptate macrospores. Any reisolate diagnosed as pure V, listed in table 8, was proved for purity by microscopic examination. Finding of any septate spores was considered sufficient to class the reisolate as impure for V, no matter what was deduced from its macroscopic appearance. The methods used were the same as those employed before, except that inoculated Marglobe plants, and likewise, Bonny Best plants inoculated with M, were grown for longer periods so that a weak infecting

organism or infections held in check by resistant plants, had ample opportunity to develop to a point at least slightly above the cotyledonary node.

Examination of the data (Table 8) from plants inoculated with mixed M and V isolates showed that in reisolations from hypocotyls of Bonny Best plants, the cultures recovered were about equally divided between those predominantly V and those predominantly M. In reisolations from hypocotyls of Marglobe plants, nearly half were impure (for type) cultures of

TABLE 8.—*Comparison of reisolates on agar from Bonny Best and Marglobe tomatoes inoculated with a mixture of virulent (V) and mild (M) types of wilt Fusarium*

Host plants	Isolates[a]	Reisolation from plant parts indicated			
		Hypocotyl[b]		Epicotyl[b]	
		Pure cultures (type observed) M/V	Mixed cultures (predominant type) M/V	Pure cultures (type observed) M/V	Mixed cultures (predominant type) M/V
Bonny Best	VR5 + MA15	0/0	6/4	2/3	4/1
	VR5 + M1–a	0/0	8/2	0/1	7/2
	VR5 + M3–a	0/0	4/6	1/2	1/6
	V7–a + M5–a	0/2	2/6	1/2	3/4
	VR5–a + M8–a	0/0	8/2	0/1	0/9
	V10–a + M10–a	0/0	3/7	0/0	4/6
	V1–a + M2–a	0/0	6/4	0/0	4/6
	V7–a + MA15	0/0	2/8	1/3	4/2
Marglobe	VR5 + MA15	0/1	3/5	0/9	0/1
	VR5 + M1–a	1/3	2/4	0/10	0/0
	VR5 + M3–a	1/4	4/1	0/9	0/1
	V7–a + M5–a	0/5	0/5	0/10	0/0
	VR5–a + M8–a	0/0	3/7	0/6	0/4
	V10–a + M10–a	0/1	0/9	0/10	0/0
	V1–a + M2–a	1/4	2/3	0/10	0/0
	V7–a + MA15	1/5	1/3	0/9	0/1
Bonny Best	VR5[c]	0/10	0/0	0/10	0/0
	MA15[c]	10/0	0/0	10/0	0/0
Marglobe	VR5[c]	0/10	0/0	0/10	0/0
	MA15[c]	10/0	0/0	10/0	0/0

[a] The same isolates as those used in table 7, except those marked a, indicate reselection.
[b] Hypocotyl = taproot, transition region, and point just below cotyledons.
Epicotyl = points just above cotyledonary node, and in base, middle, and apex of plant.
[c] Included for reference purposes.

predominantly V type, and about 30 per cent were apparently pure V. In diseased epicotyl tissues, from just above cotyledonary nodes and up into the apices of the seedlings, the reisolations from Bonny Best plants were about 15 per cent pure V. Practically all the rest were impure for type, with about equal division between those predominantly V and those predominantly M. In comparable reisolations from Marglobe plants, over 90 per cent of the cultures were pure V type, which indicated common supersedence of the M type by the V as it invaded the upper parts of the more resistant host.

DISCUSSION

These studies indicate methods whereby Fusarium isolates of highly

pathogenic nature may be obtained for use in wilt-resistance or other experimental research.

Where relative differences in disease effects are to be considered, it has been found essential that only well-purified isolates of the highest pathogenicity be employed. In the past it often has seemed advisable to use many widely different types of cultures of a pathogen in testing for disease resistance. These cultures usually were grown separately and mixed together for inoculum in the belief that a disease resistance reading, that is, the relative pathogenic attack by such a mixture, would be closest to the effect obtained under natural field conditions. The writer has concluded, however, that such mixing often may complicate results, as shown by examples given in the present paper. Such end data may be found to be full of distracting irregularities. When a number of variants of the organism are to be used simultaneously for general host reaction, they should not be grown separately and mixed just before the inoculum is applied, but should be grown together in combined culture and then used as inoculum. It would appear that a considerable advantage might be gained in the tomato wilt-resistance program by use of controlled inoculum from a single carefully purified Fusarium isolate.

Selection of an isolate should be thoroughly considered in comparison with other members, since study of widely divergent types of *Fusarium bulbigenum* var. *lycopersici* show that their characters may extend beyond the description given by Wollenweber and Reinking (17). This also has been observed by other workers (10), and one purpose of the writer's studies has been a better understanding of the wide pathogenic and cultural variability of the pathogen.

Earlier research already has disclosed (14) certain differences in the physiology of mild and virulent isolates of the tomato-wilt Fusarium. In the present work it has seemed that the virulent type of the organism is in several respects the most hardy and the most vigorous. In comparative tests of the viability of cultures of the mild and virulent isolates aged for about 2 years, most of the conidia died, but more failed to germinate from the mild isolate than from the virulent. On agar, the mild organism grew more rapidly than did the virulent, but where there was intimate competitive action through mixed growth of the two forms in liquid media, the virulent soon dominated the mild. In comparisons of thermal death points of conidia from mild and virulent isolates, the latter withstood a distinctly higher degree of heat. When susceptible Bonny Best tomato plants were inoculated with mixtures of mild and virulent types of the organism, both forms grew well up into diseased plants, but the virulent became the more abundant. When more resistant Marglobe plants were thus inoculated both forms of the fungus infected the plants, but the invasive capacity of the virulent type was so much greater that it commonly superseded the mild by the time it had reached the upper parts of these plants. All these observations indicate the much greater competitive capacity of the virulent as compared with the mild type of the wilt organism.

Variation towards mildness or virulence did not tend in either "direction" permanently. While the real nature of such reversible modification remains obscure, it would not appear to be a true mutation but rather a matter of adaptive variation. The modifications were seemingly enhanced by selective action under cultural manipulations, or the environment encountered in the host. Such adaptive variation may occur in nature and at times may result in a predominance of the more pathogenic type of organism in the field. It appears that adaptation towards increasing virulence might follow prolonged culture of resistant tomato varieties in soils infested with variant parasites that originally may have been of rather mild type.

During the past several years there have been recurrent reports (3, 6, 7, 8) of localized instances of serious wilt damage in tomatoes. These have occurred in spite of the use of the available commercial wilt-resistant or tolerant varieties. It is recognized that considerable variations in environment as well as accidental mixtures in host strains, may contribute to such apparent wilt damages as are cited. However, it is believed the studies here reported suggest a further explanation of unexpected losses to growers. Small numbers of highly virulent types of the Fusarium may develop through variant action. These being most effective competitors, would grow more successfully than the mild types in fields planted with highly tolerant or resistant hosts. The virulent type of the organism would multiply successfully in these plants, whereas the mild would be more or less held in check. On decay of the diseased plants increasingly larger quantities of spores of the virulent type of pathogen would be left in the soil. Other natural means, such as desiccation and heat, also might tend to further reduce the numbers of the milder types of the organism. The quantity of highly virulent forms in the soil might thereby increase and eventually be the cause of unexpectedly severe attack on plants otherwise known as commonly resistant to wilt.

Indeed, it would appear that the future permanence of a tomato industry reasonably free from wilt losses may depend on the continuing effort of plant breeders. They may well have to improve further the resistance of the tomato strains beyond what is now available, testing their crop varieties by inoculation with the most virulent types of the organism obtainable.

SUMMARY

By far the greatest proportion of saltations in agar cultures of the tomato-wilt Fusarium (*F. bulbigenum* var. *lycopersici*) showed a lower degree of virulence than the parent cultures. Occasional variation in pathogenicity did take place in the reverse direction, although less than one sector in a thousand from mild cultures was of a distinctly higher virulent type.

The thermal death point of conidia from a virulent form of the organism was approximately 60° C., whereas that for a mild form was a little more than 50° C. With cultures that were 2 to 2½ years old, more conidia germinated from virulent isolates than from mild. Cultures from

surviving conidia from old cultures of mild types of the organism were mostly of higher pathogenic type than those from conidia that had not been allowed to age.

On agar surfaces the mild type of the organism grew faster than the virulent, but, grown as a mixture in liquid media in the same flask, the virulent was commonly predominant over the mild.

Susceptible host plants, under the conditions imposed, were ordinarily invaded by the mild form of the organism only up to the cotyledonary node. However, when plants were simultaneously inoculated with both a mild and a virulent form, the mild type proceeded parallel with the virulent invader into the upper parts of the plant. In these plants, however, the virulent form developed more extensively than the mild. In resistant hosts the mild isolates were more confined to the basal portions of the plant, even when there was a mixed infection. Also the virulent form was commonly precedent over the mild as it extended farther up towards the plant apex.

Changes in virulence were concluded to be in the nature of adaptive variation rather than true mutation.

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OCCURRENCE, IDENTIFICATION, AND SPECIES VALIDITY OF THE BARLEY LOOSE SMUTS, USTILAGO NUDA, U. NIGRA AND U. MEDIANS

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INTRODUCTION

It has long been accepted that loose smut of barley is caused by *Ustilago nuda* Rostr., a floral-infecting fungus, which produces a deep internal infection of the seed practically beyond control by any simple treatment. About 20 years ago it was found (35) that, not infrequently, high infection could be produced by inoculating the seed as well as the flowers, and also (21, 22) that seed treatment with certain easily-applied surface disinfectants often resulted in excellent control. The discovery (29) of the seedling-infecting *U. nigra* and (30) that loose smut caused by it could be completely controlled by applying surface disinfectants to the seed seemed to explain this discrepancy.

The discovery of *Ustilago nigra*, however, raised certain questions regarding the identity and species validity of *U. nuda*, *U. nigra* and a third fungus said to be responsible for loose smut of barley, *U. medians*, as described by Biedenkopf (4). Experiments, therefore, were undertaken to determine the number and prevalence of species causing loose smut of barley in the United States, and whether the different species could be recognized by a microscopic examination and simple spore germination tests.

EARLIER RESEARCH ON CHLAMYDOSPORE GERMINATION OF BARLEY LOOSE-SMUT FUNGI

During the 50 years since *Ustilago nuda* was described, its spore-germination and other characters have been observed extensively in laboratories throughout the world (3, 6, 13, 19, 28, 33). All reports fully agree that it produces the loose type of smutted head and that its chlamydospores are echinulate and, on germinating, normally produce only branching filaments (Fig. 1, A). According to Hüttig (16), *U. nuda* may be induced to form sporidia by germinating the spores at freezing temperatures. Thren (34), however, found that its promycelial cells separated under these conditions but formed no sporidia. It would seem probable, therefore, that the type of spore germination of *U. nuda* is sufficiently fixed to serve a useful purpose in species identification.

Ustilago nigra, also, is an echinulate-spore fungus. The chlamydospores, however, normally germinate by producing a promycelium bearing typically 4 lateral sporidia (30) (Figs. 1, B and 2, A). Referring to the "third

[1] The writer is indebted to M. A. McCall and A. G. Johnson, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for helpful advice regarding nomenclature and procedure in the present study. The writer is also indebted to Edith K. Cash for preparing the Latin description of *Ustilago nigra*.

species of barley smut known as *Ustilago nigra* or *Ustilago medians*," Allison (2) also reported that it consistently formed sporidia on potato-dextrose agar. Ruttle (25) described several intermediate barley smuts, similar to *U. nigra*, that produced sporidia. In studies of *U. nigra* on grasses, Fischer (10) germinated the chlamydospores on potato-dextrose

FIG. 1. The three types of spore germination obtained on potato-dextrose agar at 20° C., in the study of 500 barley loose-smut collections from different parts of the United States. A. The mycelial type of *Ustilago nuda*. B. The sporidial type of *U. nigra*. C. The mixed mycelial-sporidial type of *U. medians* that proved to be a mixture of *U. nuda* and *U. nigra*. ×275.

agar and obtained sporidia, as with *U. hordei*. This is good agreement and indicates that it should not be difficult to distinguish *U. nigra* from *U. nuda* through difference in type of spore germination.

Ustilago medians was described as forming echinulate chlamydospores that germinated in a most unique manner. When sown on gelatin, some produced the mycelial germination typical of *U. nuda;* but the majority

formed a promycelium and sporidia typical of *U. hordei* (Fig. 1, C). As noted by Biedenkopf (4): "Neben solchen Kolonien, die sich als von der nur Mycel bildenden *Ustilago Hordei* ansprechen liessen, fanden sich jedoch auch in grösserer Zahl solche, wie sie sich von Conidien bildenden Ustilagineen zu bilden pflegen."[2]

Suspecting that he might have in one smutted head a mixture of the echinulate-spore, mycelium-producing loose smut (*Ustilago nuda*) and the smooth-spore, sporidia-producing covered smut (*U. hordei*) ("ob in diesem Fall *Ustilago Jensenii* und *Ustilago Hordei* nicht etwa zusammen auf einer Ähre sich fänden"),[2] Biedenkopf examined the spores, but found none of covered smut in the loose-smut heads, because all of the spores were echinulate. In the second and final test the spores were sown in diluted plum decoction and germinated within 4 hours. Again, both the conidial and mycelial types of germination occurred. As noted by Biedenkopf: "Einige trieben einen meist dreizelligen Mycelschlauch und begannen nun in rascher Folge Conidien abzuschnüren, die sofort nach dem Abfallen in hefeartige Sprossung übergingen. Die meisten Sporen dagegen keimten zu Mycelien aus." In the first test, therefore, most of the spores formed sporidia but in the second test most of the spores formed mycelium.

Further observation of the cultures showed that the later developments of the mycelial and sporidial types of germination were identical with those normally characteristic of *Ustilago hordei* Bref.[3] and *U. jensenii* Rostr.[3]

Biedenkopf's excellent descriptions leave no doubt but that his *Ustilago medians* was an echinulate-spore loose smut of barley with a mixed mycelial-sporidial type of spore germination, similar to or identical with that produced by a mixture of *U. nuda* and *U. hordei*. Therefore, if *U. nuda*, *U. nigra* and *U. medians* have been correctly described, the morphological character that should distinguish the three species is the mycelial germination of the spores of *U. nuda*, the sporidial germination of those of *U. nigra* and the mixture of mycelial and sporidial types produced by the chlamydospores of *U. medians*.

MATERIAL AND METHODS

The loose smut of barley here reported comprised 500 viable collections from the following States: Arkansas, Colorado, Delaware, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maryland, Michigan, Minnesota, Mississippi, Missouri, Montana, Nebraska, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Virginia, Washington, West Virginia, Wisconsin, and Wyoming.

Past research on the floral-infecting loose smut (*Ustilago nuda*) frequently has been hampered by its short-lived spores. This difficulty was met in the present study by storing the *U. nuda* and other collections at

[2] Biedenkopf applied the binomials *Ustilago hordei* Brefeld to the loose smut (*U. nuda* Rostr.) and *U. jensenii* Rostrup to the covered smut (*U. hordei* Lagerh.).

[3] See footnote 2.

about 1° C. The formulae of Riker and Riker (23) were used in the preparation of all media for spore-germination tests, excepting the malt agar, which was made according to Fischer's (11) formula No. 5.

In the study of the occurrence of species of barley loose smut in the United States, as indicated by epispore and spore-germination characters (p. 197), the former were determined by examination under an oil-immersion lens. Spore-germination characters were determined by sowing the spores on fresh 2 per cent potato-dextrose agar in 50-mm. Petri dishes. The cultures were then incubated at 20° C. in a constant-temperature chamber, and examined after 6 to 12 hours. The spores were sown according to a previously-described method (33) whereby individual spores in aqueous suspension were uniformly distributed over the agar (Fig. 1). Every spore was placed in direct contact with the exposed surface of the substrate where it could be accurately observed for type of germination. It was observed, for example, that before this method was developed, if a small clump of dry spores of *Ustilago hordei* or *U. nigra* were placed on potato-dextrose agar, the spores touching the medium produced sporidia; those piled on other spores, however, sometimes germinated by producing only mycelial threads. The two different conditions involved were responsible for this, but the spores mistakenly presented a mixed germination on one medium. The technique of setting up the cultures, therefore, is an important consideration in an accurate species determination.

EXPERIMENTAL RESULTS

Groups of Barley Loose Smut in the United States

As noted previously (p. 196), *Ustilago nuda, U. nigra,* and the so-called *U. medians* have in common the production of the loose type of smutted head and of echinulate chlamydospores. The morphological character distinguishing the three species is the mycelial germination of the chlamydospores of *U. nuda,* the sporidial germination of those of *U. nigra,* and the mixture of mycelial and sporidial types produced by the chlamydospores of *U. medians.* Heretofore, however, it has been difficult to judge the distribution and importance of these species in this country because, in some of the articles, the binomial *U. medians* clearly was used not in reference to a smut with the mixed *U. nuda-U. hordei* type of germination, as described for that species, but to one with the sporidial type of germination. In other reports, the implications of this binomial are not given, and it may or may not have been used in compliance with Biedenkopf's description. As a result of the investigation here recorded, the fungi of the 500 loose-smut collections examined were assignable to 4 well-defined groups on the basis of (1) the type of smutted heads, (2) epispore markings, and (3) type of spore-germination on 2 per cent potato-dextrose agar at 20° C. The defining characters and number of collections found in each group follows:

Group 1.—Loose-smut heads; echinulate spores that produce only mycelial threads (Fig. 1, A). 192 collections. This is the type of smutted head, epispore

marking, and chlamydospore germination described for *Ustilago nuda* Rostrup (24).

Group 2.—Loose-smut heads; echinulate spores that produce only promycelia and sporidia (Figs. 1, B, 2 and 4). 209 collections. This is the type of smutted head, epispore marking, and chlamydospore germination described for *Ustilago nigra* Tapke (29, 30).

Group 3.—Loose-smut heads; echinulate spores. An indefinite number of the spores produce mycelial threads, while the remainder simultaneously produce promycelia and sporidia (Fig. 1, C). 93 collections. This is the type of smutted head, epispore markings, and chlamydospore germination described for *Ustilago medians* by Biedenkopf (4).

Group 4.—Heterogeneous forms having a mixture or blending of characters of smutted heads, or epispore markings of *Ustilago nigra* and *U. hordei* Lagerh.[4] Spores produce only promycelia and sporidia. 6 collections. The existence of this small group must be recognized, but the variability in smutted head and epispore characters hardly permits taxonomic treatment. They probably are hybrid forms.

Of the 500 collections studied, 192 were pure *Ustilago nuda* (group 1), and 209 were pure *U. nigra* (group 2). Chlamydospores of each of the 209 collections of *U. nigra* were applied to the surface of seed of Odessa (C.I. 934) barley. This variety proved to be highly susceptible to every collection. All of these collections, therefore, have the characters described for *U. nigra* in the original (29) and supplemental (30) descriptions of this species, *i.e.*, loose-smut heads, echinulate spores that germinate with sporidia, and the ability to produce seedling infection. The 93 collections of group 3 were studied further and the results are given below.

The Composition of Collections Complying with the Description of Ustilago medians Biedenkopf (Group 3)

A mixture of the normal mycelial and sporidial germinations occurred in each of the 93 collections that appeared to be *Ustilago medians*. Both types developed with great vigor in 6 to 12 hours and complied with the mixed "*U. jensenii-U. hordei*" type of germination described by Biedenkopf (4) for *U. medians*. The proportion of the two types varied widely in the different collections, ranging from a trace of one or the other to approximately equal proportions of each. Further examination of these 93 (group 3 or *U. medians*) collections, revealed that each was simply a mixture of the sporidia-producing *U. nigra* and the mycelium-producing *U. nuda*, rather than an intermediate species. This was proved conclusively in 3 ways: (1) Plants of the smut-susceptible Odessa (C.I. 934) barley were grown from seed coated with chlamydospores of each of the 93 mixed collections. This procedure filtered out the *U. nuda* element due to its inability to produce seedling infection. The *U. nigra* element of each collection, however, produced high percentages of smutted heads whose spores invariably produced only the sporidial type of germination; (2) flowers of Lion (C.I. 923) and Odessa (C.I. 934) barley, both of which are susceptible to *U. nuda* and *U. nigra*, were hand-inoculated with each of 10 of the collections from group 3. On maturing, some of the seed was sown without treatment; another lot was

[4] *Ustilago hordei* Lagerh. (*U. hordei* (Pers.) K. and S.) produces the covered type of smutted head, has smooth chlamydospores, which produce sporidia on 2 per cent potato-dextrose agar at 20° C.

soaked in formaldehyde solution (1:320) for 1 hour before sowing. This treatment does not control *U. nuda*, but it does control *U. nigra* (30). The plants grown from treated seed produced only *U. nuda*, the spores of which yielded only the mycelial type of germination. Plants from the nontreated seed of Lion and Odessa, however, became infected by both *U. nuda* and *U. nigra;* (3) spores of 10 of the mixed collections of group 3 were inserted in the flowers of Atlas (C.I. 4118) and Trebi (C.I. 936) barleys. In the writer's studies, Atlas has proved immune from *Ustilago nigra* and highly susceptible to *U. nuda*. Trebi, however, has been immune from *U. nuda*, but highly susceptible to *U. nigra*. The mature seed from inoculated flowers of both varieties was later sown in a greenhouse. From the smutted heads of Atlas plants only *U. nuda* was recovered, and from Trebi only *U. nigra*. Both *U. nuda* and *U. nigra* have been isolated many times from naturally mixed collections by the foregoing methods and then used separately year after year in studies of physiologic races and in other experiments. Such isolates have never shown evidence of the simultaneous mixed type of spore-germination described by Biedenkopf for *U. medians*.

In the light of the foregoing results, it would seem that Biedenkopf's *Ustilago medians* may have been similar to the numerous field collections of barley loose smut reported herein that proved to be mixtures of *U. nuda* and *U. nigra* and that germinated in full conformity with his description of *U. medians*. It is noteworthy, too, that in germinating random samples of mixtures of *U. nuda* and *U. nigra*, more of either one may be taken in successive samples. The sporidial germination, therefore, may predominate in one sample and the mycelial in the next. Biedenkopf reported results of this kind in his two tests of *U. medians*. Apparently, no other smut has been reported that persistently develops the normal mycelial and sporidial types of germination simultaneously in one culture. No further specimens of *U. medians* as described by Biedenkopf evidently have been found anywhere.

It would seem unsafe to assume that the sporidial element of *Ustilago medians* was identical with *U. nigra;* in fact, the sporidial element may not have been a loose smut. For example, the writer has collected an echinulate-spore, sporidia-forming, barley smut similar to *U. nigra*, except that during the past 5 years it has consistently produced the covered-smut type of head. Spores of this smut were dusted on the surface of Odessa (C.I. 934) barley seed. Earlier, the flowers from which this seed developed were hand-inoculated with spores of the deep-infecting loose smut, *U. nuda*. Plants from this twice-inoculated seed, grown in a greenhouse, produced many smutted heads of different kinds. Several of the loose-smut heads evidently had some admixture of the echinulate-spore covered smut because some spores formed sporidia, while others formed mycelium on 2 per cent potato-dextrose agar at 20° C. The loose-smut heads, echinulate chlamydospores, and mixed types of spore germination, therefore, were like *U. medians*, but the sporidial element in this mixture was not the loose-smut fungus *U. nigra*.

Another possible explanation of Biedenkopf's description might be that his smut was a sporidia-producing species that in both of his tests germinated abnormally to resemble the mixed mycelial-sporidial type. This phase is considered in the following pages.

Variability in Spore-germination

Commenting on the species validity of *Ustilago medians* and *U. nigra*, Ruttle (25) called attention to the fact that Biedenkopf's germination studies were performed in the heat of July "under which conditions even spores of *U. hordei* may germinate by germ tubes." It is well known that so-called sporidia-producing smuts, *e.g.*, *U. hordei*, do not, on germinating, always produce sporidia; but, in the light of the studies of Hüttig (16), Jones (17, 18), Shih (27), and numerous others, it is equally true that they do not simultaneously produce both the sporidial and mycelial types of germination in optimum development under one set of conditions in culture, as described by Biedenkopf for *U. medians*. However, in view of the relatively limited knowledge of spore germination in *U. nigra*, a study of this character as influenced by different temperatures and substrata was included in the present investigation.

In the temperature studies, spores were sown by a method previously described (33) on the surface of 2 per cent potato-dextrose agar in 50-mm. Petri dishes and incubated in accurately controlled temperature chambers at 5, 10, 15, 20, 25, 30, 35, and 40° C. Three chlamydospore cultures of each of 3 physiologic races of *Ustilago nigra* were incubated at each temperature. The spores were nearly 100 per cent viable. For comparison, the 3 well-known sporidia-producing small-grain smuts, *U. avenae*, *U. levis*, and *U. hordei* were similarly studied. The results with *U. nigra* were as follows: At 5, 10, 15, 20, and 25 degrees C. all of the viable spores germinated with short tubes and sporidia. At 30° the temperature was obviously too high for normal germination, and the spores uniformly developed short, thick, segmented threads resembling links of sausage. At 35°, only short tubes were formed, and at 40° no germination occurred. The results with *U. avenae*, *U. levis*, and *U. hordei* were similar to those with *U. nigra* throughout the test. Spore germination in *U. nigra* at various temperatures, therefore, is strikingly like that of *U. avenae*, *U. levis*, and *U. hordei*, and unlike that described by Biedenkopf for *U. medians*.

In the study of variability in type of spore germination, as influenced by different substrata, the same collections and species used in the foregoing study were again employed. Each was tested in triplicate cultures at 20° C. on the following substrata: 1, On the lower surface of bare glass cover slips suspended on glass bars above a moist blotter in a moisture chamber; 2, in sterile distilled water; 3, in sterile tap water; 4, on plain gelatin; 5, on nutrient gelatin; 6, on 2 per cent potato-dextrose agar; 7, on nutrient agar; 8, on malt agar; 9, on prune agar; 10, on plain distilled-water agar.

Throughout the test, spore germination was strikingly similar in *Ustilago*

avenae, *U. levis*, *U. hordei*, and the 3 collections of *U. nigra*. The results, moreover, agreed fully with those of Brefeld (5), Hallier (12), Fischer (10), Kulkarni (20), Sartoris (26), Shih (27), Stakman (28), and others in sim-

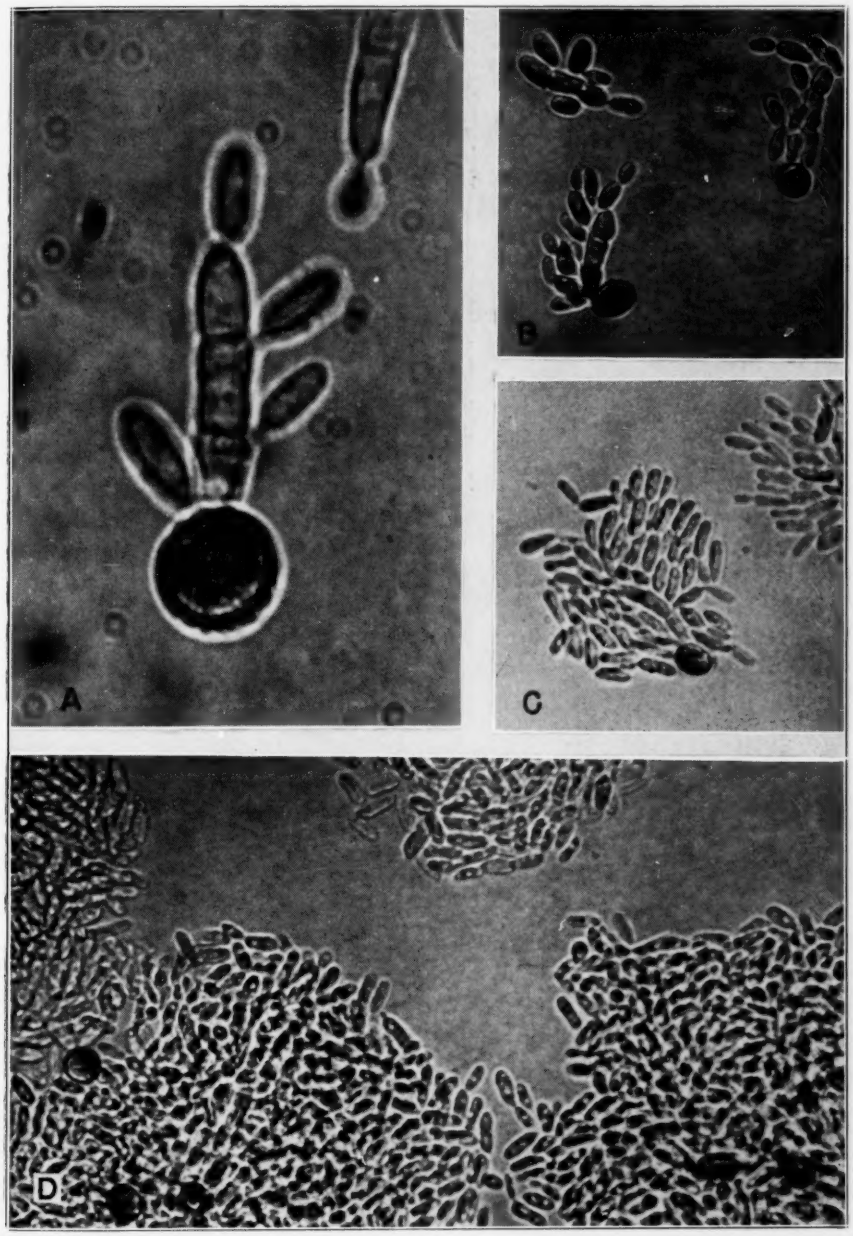

FIG. 2. Germination of spores of *Ustilago nigra* on 2 per cent nutrient-dextrose agar at 20° C. A. An early stage showing the echinulate chlamydospore, biseptate promycelium and four primary sporidia. ×1900. Another promycelium, bearing a young primary sporidium at its apex is shown in the upper-right corner. B, C, and D. Later stages of germination showing multiplication of sporidia. ×600.

ilar studies of various sporidial smuts. On potato-dextrose, nutrient-dextrose, and malt agars the 4 species formed only sporidia. As shown in figure 2, the promycelia were compact and sturdy, and sporidia were produced in countless numbers. On the other hand, when spores of these smuts were

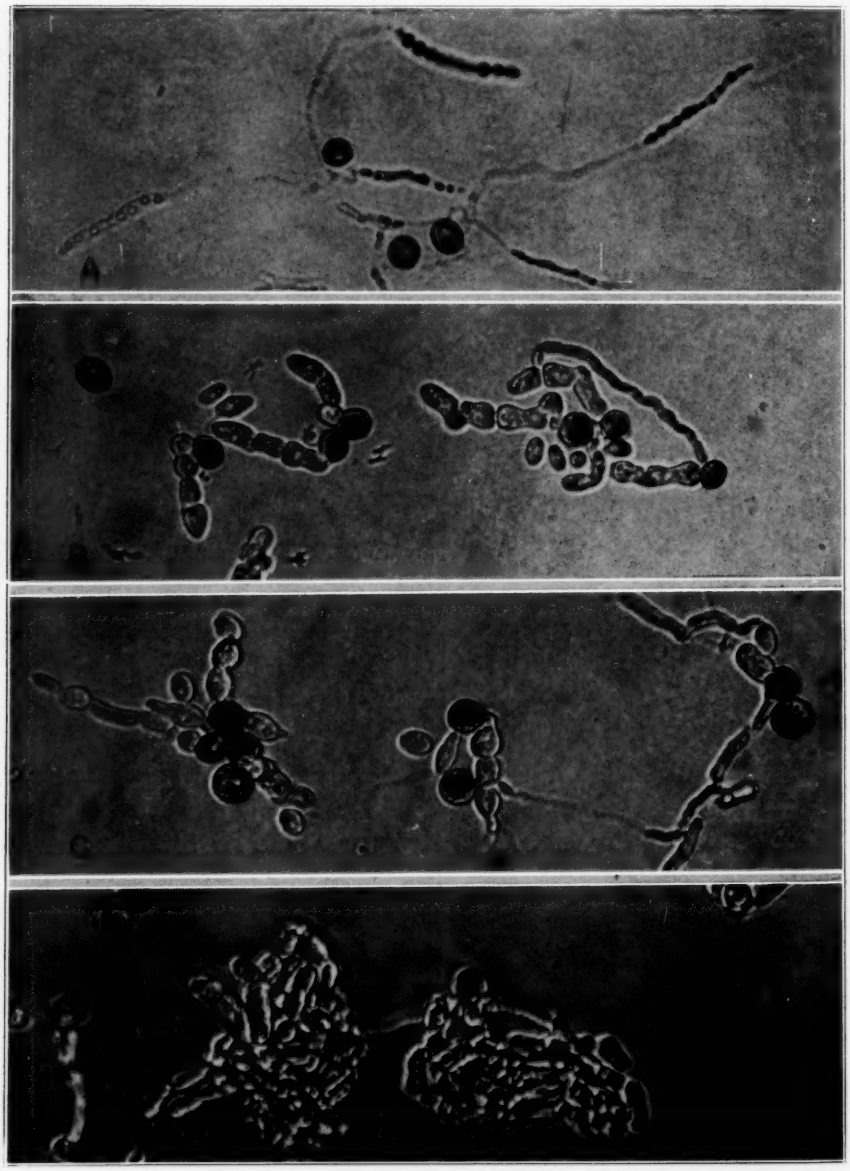

FIG. 3. Examples of the influence of different conditions on spore germination of *Ustilago avenae, U. hordei, U. levis* and *U. nigra.* A. *U. levis* on a bare glass cover slip in humid air. B. *U. hordei* on distilled-water agar. C. *U. nigra* on distilled-water agar. D. *U. avenae* on plain gelatin. All photographs taken after 5 days in culture at 20° C. × 600.

FIG. 4. A. An unsmutted head of Odessa (C.I. 934) barley. B, C, E. Odessa heads smutted with *Ustilago nigra* as they appeared, respectively, 1 day after emergence, when the barley was in the milk stage, and when the barley was ripe. D. A smutted head of Himalaya (C.I. 1312) barley showing the greater persistence of awns in smutted heads of this strongly awned variety. F. Smutted head of the hooded, hulless, Nepal (C.I. 595) barley. The distinguishing head characters of different barley species and varieties are largely lost when the heads become smutted. ×1.

germinated in the absence of nutriment on bare glass cover slips in a moisture chamber, only mycelial threads were produced (Fig. 3, A). Prune agar was fairly favorable for the production of sporidia. Plain and nutrient gelatins were clearly unfavorable. The spores gave rise mostly to abortive, compact masses of cells (Fig. 4, D). Biedenkopf used gelatin and diluted plum decoction in the study of spore germination in *U. medians,* but did not give the compositions of these media. It is evident, however, that on plain gelatin, nutrient gelatin, and prune agar, spore germination in *U. nigra* bears no resemblance to the mixed mycelial-sporidial type (Fig. 1, C) described by Biedenkopf for *U. medians.* Distilled-water agar also was unfavorable (Fig. 3, B, C). On this medium, and also in tap water and distilled water, some spores produced a few sporidia, others a few short threads, while still others developed a third type, not mentioned by Biedenkopf, usually consisting of two branches, one bearing a few sporidia, the other forming an abortive mycelium-like growth. It was thus possible to produce in *U. nigra, U. hordei, U. avenae,* and *U. levis* an abnormal or atypical sporidial germination that might be taken to resemble a mixture of the mycelial and sporidial types accompanied by a third type, as described above. This germination, however, was very slow, and, after an abortive development, came to a standstill. Cultures several weeks old still showed very scant growth. In morphology and rate and degree of development, therefore, this abnormal sporidial type unmistakably was not the luxuriant development of robust mycelial threads and thousands of sporidia, budding like yeast, that Biedenkopf described for *U. medians,* and first suspected to be "*Ustilago Hordei* und *Ustilago Jensenii* . . . zusammen" (4). It should be noted, moreover, that Biedenkopf obtained the mixed mycelial-sporidial germination of *U. medians* in only 4 hours. It is evident from studies of other small grain smuts (3, 16, 27) that this is a very rapid rate, indicating that optimum or near optimum temperatures existed during germination. At such temperatures and on favorable substrata such as Biedenkopf evidently used, neither a mycelial nor a sporidial smut alone would develop the mixed mycelial-sporidial type of germination described for *U. medians* (4). But, granted even that *U. medians* had been based on the abnormal germination of a sporidial smut, the species name would have to be rejected in compliance with Article 65 of the International Rules of Botanical Nomenclature (7), which reads "A name or epithet must be rejected when it is based on a monstrosity."

In view of the foregoing results with different temperatures and substrata, it is evident that, even when variability in spore germination is given the widest latitude, the persistent, normal development of two distinctly different types of spore germination with which Biedenkopf characterized *Ustilago medians,* does not occur in *U. nigra* and other sporidial smuts. *Ustilago nigra* and *U. medians,* therefore, are not synonymous species.

As shown recently by Christensen and Rodenhiser (8), the genetic makeup of the chlamydospore, as well as its environment, may be concerned

in variability of chlamydospore germination. In *Ustilago zeae* and *U. levis*, for example, certain lines consistently produce the mycelial instead of the normal sporidial type of germination. In *U. nigra*, however, such lines apparently have not been found or reported, although many specimens of the fungus have been studied in recent years.

Variability in Ustilago nigra

Variation occurs in *Ustilago nigra* in prominence of the chlamydospore echinulations, intensity of the dark brown color of the spore mass, compactness and powderiness of the smutted heads, degree to which the fungus destroys the awns of smutted heads (Fig. 4) etc. The host variety, physiologic race of the smut, and the conditions under which the plants are grown seem largely responsible for these variations (32). Similar or greater variability than that yet found in *U. nigra* has been reported in such well-known and long-established species as *U. avenae* and *U. levis* (14), *U. hordei* (32), *Sphacelotheca sorghi* and *S. cruenta* (36), *Tilletia tritici* and *T. levis* (15), and others (8). Ruttle (25) erected "types" of barley smut on small morphological differences like those noted above for *U. nigra*, but they fail to breed true and apparently serve no useful taxonomic purpose.

Conclusions and Amplified Description of Ustilago nigra

In the light of the foregoing facts, the following conclusions seem logical:

1. The so-called *Ustilago medians* evidently does not occur in the United States. *Ustilago nuda* and *U. nigra*, however, frequently occur together in the barley fields of this country and a mixture of the spores of these two species germinates by producing the mixture of mycelial and sporidial types, as described for *U. medians*, indicating that the latter probably was based on a mixture. 2. Excepting occasional hybrid types, loose smut of barley in this country is caused only by *U. nuda* and *U. nigra*. 3. The incidence of species in the present study indicates that *Ustilago nigra* has become as widespread as *U. nuda* in the United States. It is, therefore, now possible through simple, inexpensive seed treatments, to save half of the estimated loss from loose smut averaging 2 million bushels of barley annually (29). 4. When *U. nuda* and *U. nigra* are uniformly germinated on 2 per cent potato-dextrose agar at 20° C., *U. nuda* develops only the mycelial type of germination and *U. nigra* only the sporidial type. These 2 species, therefore, always may be readily and accurately identified by the striking differences in this character (Fig. 1, A and B). 5. The normal germination of *U. nigra* is consistently that of a true sporidial smut; it positively does not resemble "*Ustilago Hordei* and *Ustilago Jensenii* . . . zusammen,"[5] as described for *U. medians* by Biedenkopf (4). 6. Through the use of different temperatures or different substrata, chlamydospores of *U. nigra*, like those of *U. avenae*, *U. levis*, and *U. hordei* may, on germination in culture, produce only sporidia, or mycelia only, or a simultaneous development of abortive forms

[5] See footnote 2.

of both sporidia and mycelia. The simultaneous, optimum, development of both the mycelial and sporidial types in one culture, as described by Biedenkopf, for *U. medians* did not occur. 7. In view of these facts it is evident that, even when variability in spore germination is given the widest latitude, identity of *U. nigra* is distinct from that of the mycelial-sporidial *U. medians*. *Ustilago nigra*, therefore, is entitled to rank as a species. 8. The binomial *U. medians* Biedenkopf must be rejected (A) because it was obviously based on a mixture of two different fungi, erroneously supposed to form part of one individual; (B) because it cannot be applied with certainty to any known fungus causing loose smut of barley; and (C) because the binomial has become a source of confusion or error due to its use with different meanings. It, therefore, falls clearly within the scope of articles 64, 63, and 62 of the International Rules of Botanical Nomenclature (7), which are as follows: Article 64.—"A name of a taxonomic group must be rejected if the characters of that group were derived from two or more entirely discordant elements, especially if those elements were erroneously supposed to form part of the same individual" (*Nomina confusa*). Article 63.—"A name of a taxonomic group must be rejected when its application is uncertain" (*Nomen dubium*). Article 62.—"A name of a taxonomic group must be rejected if owing to its use with different meanings it becomes a permanent source of confusion or error" (*Nomina ambigua*).

Amplified Description of Ustilago nigra

Ustilago nigra was briefly described in 1932 (29), and further described in 1935 (30) and in 1941 (33). In order to summarize these descriptions, the following amplified description is presented:

Ustilago nigra Tapke. Sori in spikelets forming dusty, dark-brown spore masses 3–5 × 5–10 mm. Sori temporarily protected by a thin membrane, but soon becoming dissipated, leaving the naked rachis (Fig. 4, B, C, E). Spores echinulate, of lighter color on one side, spherical to subspherical, 5.5–7.5 × 6–8 μ, mostly 6.5 × 7 μ, forming a 3-cellular promycelium bearing typically 4 lateral sporidia when germinated on 2 per cent potato-dextrose agar and similar media at about 20° C. (Fig. 2, A, B, C, D).

On *Hordeum vulgare* (31) *H. distichon* (31), *H. intermedium* (31), *H. deficiens* (31), *H. nodosum* (10), *H. pusillum*,[6] *Elymus canadensis* (10), and *Sitanion jubatum* (10). Collected in the following States: Arkansas, Colorado, Delaware, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maryland, Michigan, Minnesota, Mississippi, Missouri, Montana, Nebraska, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Virginia, Washington, West Virginia, Wisconsin, and Wyoming.

Herbarium Material: Specimens deposited in the Mycological Collections

[6] Collected by I. M. Atkins, Denton, Texas, and determined by the writer as *Ustilago nigra*, physiologic race 4.

of the Bureau of Plant Industry, United States Department of Agriculture at Washington, D. C. Type No. 71357 on *Hordeum vulgare.*

Soris in spiculis, massam pulverulentam atro-brunneam 3–5 mm. latem, 5–10 mm. longam formantibus, primum membrans tenui tectis, demum nudis; Sporis echinulatis, sphaericis v. subsphaericis 5.5–7.5 μ latis, 6–8 μ longis, plerumque 6.5 μ latis, 7 μ longis; promycelio 3-septato, sporidia 4, lateraliter gerenti.

Hab. in spiculis *Hordei* spp., *Elymi canadensis,* et *Sitanii jubati.* United States.

SUMMARY

In a study of the different kinds of barley loose smut in this country, 500 specimens collected from 33 States were uniformly germinated on 2 per cent potato-dextrose agar at 20° C. Six of the collections were heterogeneous types. Of the remainder, all of which had the loose type of smutted head and echinulate spores, 192 produced the mycelial germination of *Ustilago nuda,* 209 the sporidial germination of *U. nigra,* and 93 produced on germination a mixture of the normal mycelial and sporidial types and otherwise conformed to the description of *U. medians* Biedenkopf. All of the 93, however, proved to be simply mixtures of the mycelium-producing *U. nuda* and the sporidia-producing *U. nigra.* A smut conforming to the description of *Ustilago medians* apparently does not occur in the United States nor elsewhere.

When spores of *Ustilago nuda* and *U. nigra* are uniformly germinated on 2 per cent potato-dextrose agar or other suitable media at usual room temperatures, the former consistently develops only mycelial threads, and the latter always forms only promycelia and sporidia. The spore-germination test, therefore, quickly and accurately shows (1) whether the causal fungus is *U. nuda, U. nigra,* or a mixture of the two, and (2) the type of seed treatment necessary for control.

In a comparative study, chlamydospores of *Ustilago nigra, U. avenae, U. levis,* and *U. hordei* were germinated on 10 different substrata at 20° C. and on 2 per cent potato-dextrose agar at 5, 10, 15, 20, 25, 30, 35 and 40 degrees C. Conclusive evidence was obtained that *U. nigra* is a true sporidia-producing fungus like *U. avenae, U. levis,* and *U. hordei.*

The incidence of species in the present study indicates that *Ustilago nigra* has become as widespread as *U. nuda* in the United States. It is, therefore, possible through simple, inexpensive seed treatments, to prevent half of the estimated annual 2-million-bushel loss attributed to barley loose smut.

It is concluded that, with the exception of occasional hybrid types, such as are found also in wheat and oat smuts, loose smut of barley in the United States is caused by *Ustilago nuda* or *U. nigra;* that these two species may be readily distinguished through a simple spore-germination test and in other ways; that *U. nigra* is a valid species; and that *U. medians,* appar-

ently, was erroneously based on a mixture of two different smut fungi. The binomial, therefore, must be rejected.

An amplified description of *Ustilago nigra* is given.

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AN OUTBREAK OF PLANTAGO VIRUS IN BURLEY TOBACCO[1]

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During the meeting of the New England Division of the American Phytopathological Society in 1941, F. O. Holmes showed to one of the writers plants of *Plantago major,* which he stated were affected by a virus similar to the tobacco-mosaic virus because it withstood drying, and when purified and photographed, was indistinguishable in appearance from the tobacco-mosaic virus. The details of his study have since been published (2). He also reminded us of a report, in the 1930 Annual Report of the Kentucky Experiment Station, of a virus of *Plantago major* that we had transferred to tobacco. In these early studies a mosaic-affected *Plantago major* leaf was collected from the experiment station lawn and a transfer made to Turkish tobacco, where a disease developed, different from that produced by any of the strains of the tobacco-mosaic virus then under investigation. On seedling potato leaves the plantago virus caused chlorotic spots that soon became necrotic (3). In this respect it was similar to but not identical with tobacco-mosaic virus. It caused local chlorotic spots on petunia, with chlorosis and distortion in growing-point leaves. No infection followed inoculation of cucumber. On June Pink tomato the plantago virus caused a mild mottling which might have been easily overlooked. In addition it caused necrosis of the underside of the midveins. Dried tobacco material was saved to determine whether the virus withstood drying but this material was not tested until recently when both tobacco mosaic and plantago virus were isolated from it. On Cut-short, Striped creaseback, and White creaseback beans the plantago virus produced no necrotic spots, while tobacco-mosaic virus strains caused local necrotic spotting. Primarily because of this fact, it was concluded that the plantago virus was not a strain of tobacco-mosaic virus, and probably was of little importance to the tobacco industry. No further study of the virus was made at that time.

After returning to Lexington from the meeting, the writers examined plants of plantago and found many infected with a virus. On inoculated Ky. 16 burley this virus caused local chlorotic spots, followed eventually by necrosis of the whole inoculated area; it also caused vein-clearing of growing-point leaves, soon followed by mottling in which the dark green areas were small specks, instead of large, irregular areas, as in tobacco mosaic. There was some distortion, and necrotic spots developed in leaves or portions of leaves that were young enough to be rapidly invaded, but too old to develop mottling at the time of invasion (Fig. 1, B). The virus is similar in this respect to burning strains of the tobacco-mosaic virus (5), except that the latter rarely cause burning in the greenhouse. In older plants, growing in the ground bench, the patterns were usually larger and distortion less than

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

occurs with many strains of tobacco mosaic. On Turkish tobacco, symptoms developed similar to those recently described by Holmes (2). The plantago virus, as it occurs in Kentucky, is obviously the same as that found in eastern United States and described by Holmes.

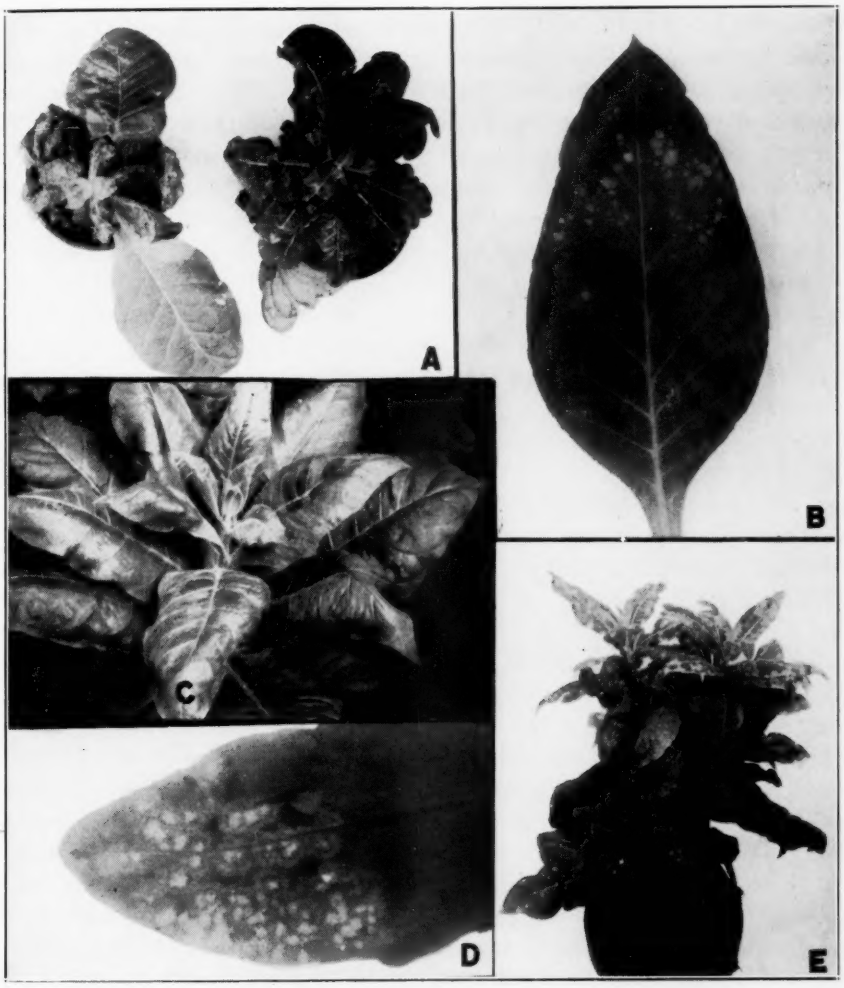

FIG. 1. A. Two N′N′ burley plants, Kelley (left) and Pepper, infected with the plantago virus from plantago. The plant on the left was inoculated 51 days later than the one on the right, and shows the severe necrotic reaction following inoculation. Photographed 36 days after the Kelley plant was inoculated. B. A leaf from a plant of Ky. 16 burley inoculated with the plantago virus, showing necrotic areas in invaded tissue too old to develop mottle, and speckled mottling in the younger portion of the leaf. C. Ky. 16 burley naturally infected with the plantago virus in the field. This shows the chlorotic response of the virus in an n′n′ plant. D. *Plantago major* leaf 19 days after inoculation with a variant of tomato aucuba mosaic virus. The patterns are somewhat more prominent than those produced by the ordinary strains of the tobacco-mosaic virus. Leaf print on direct positive paper. E. Ky. 36–12 N′N′ plant from Mr. Bell's field infected with the plantago virus, transplanted to the greenhouse in August and photographed November 6, 1941.

Late in August, 1941, R. E. Bell, a neighboring farmer, brought us several plants of Ky. 36–12 burley tobacco affected with a virus disease we did not recognize. The plants were markedly stunted; had long, depressed, dark-colored streaks on the stalks, and discolored patches in the pith which sometimes had concentric patterns. The leaves had yellow blotches, sometimes only 2 or 3 to the leaf, which later became necrotic (Fig. 1, E). Some leaves had necrotic line patterns and, on the undersides of the midveins, necrotic areas. The disease differed from tobacco streak and ring spot because there was no indication of so-called recovery in the new growth. The farmer said that the disease had been present since shortly after setting the crop. A count in the field indicated that about 2 per cent of the plants were affected but the farmer claimed this percentage was low because he had destroyed many affected plants during the summer. A crop of Ky. 5 burley, plants for which were obtained from a neighbor's bed and set after Ky. 36–12 in the same field, was entirely free from the disease, suggesting that infection of Ky. 36–12 probably originated in the plant-bed. The only disease of tobacco, similar to the Bell disease, which we had seen in the past, was one collected in Marion County, Kentucky, in 1923, and illustrated under the name "streak" in Kentucky Bulletin 328. A re-examination of records and photographs of this disease indicates that it corresponds more closely to the Bell disease than to the disease now commonly known as tobacco streak.[2]

Infected plants from the Bell field were transferred to the greenhouse where they continued to show a streak type of disease month after month (Fig. 1, E). Inoculations were made from these transplanted plants to Burley 16 and to Turkish, where a mottle, rather than a necrotic, disease developed. A comparison of these mottled plants with Turkish and Burley 16 plants inoculated from plantago, indicated that the diseases were at least very similar, and the suggestion occurred that the virus causing the streak-like disease in the field was the plantago virus in a variety of tobacco, which reacted to the virus in essentially the same way as burley tobacco plants containing the N factor from *N. glutinosa* react to any of the strains of the tobacco mosaic virus.

It had previously been shown that the varieties of burley and dark tobaccos grown in Kentucky, as well as collections from other parts of the world, could be divided into two distinct groups, based on their reaction to white tobacco mosaic, tomato aucuba mosaic, and to several other collections of the tobacco-mosaic virus (6). On one group of varieties, these viruses caused necrotic lesions in about 48 hours, and streak, if the virus became systemic. The same group inoculated with ordinary strains of the virus always developed the usual systemic mottle. The other group when inoculated with the necrotic strains of the virus develop typical mottle mosaic. The gene controlling necrotic spotting is a simple dominant, as is the similar gene in *Nicotiana glutinosa*. The necrotic spotting gene in the common tobacco varieties will be designated N′ to distinguish it from the N gene from

[2] In 1942 the Marion county farm was visited and numerous infected plantains were found bordering the field in which the disease of tobacco was found in 1923.

N. glutinosa, which has now been transferred to several varieties of tobacco. Whether or not the N and N′ genes are allelomorphs has not been determined. Burley tobacco varieties in which necrotic spotting genotypes occur, and which will be designated as N′ burleys, are Kelley, Judy's Pride, Pepper, and Ky. 36–12. Dark varieties that contain the N′ factor are One Sucker, Improved Smith, Rudolph's Improved Mammoth, Madole, Brownleaf, and Ambalema. The following varieties develop typical mosaic when inoculated with the necrotic strains of the virus: Turkish, Ky. 5, Ky. 16, Gay's Yellow, and Chileno Corentino.

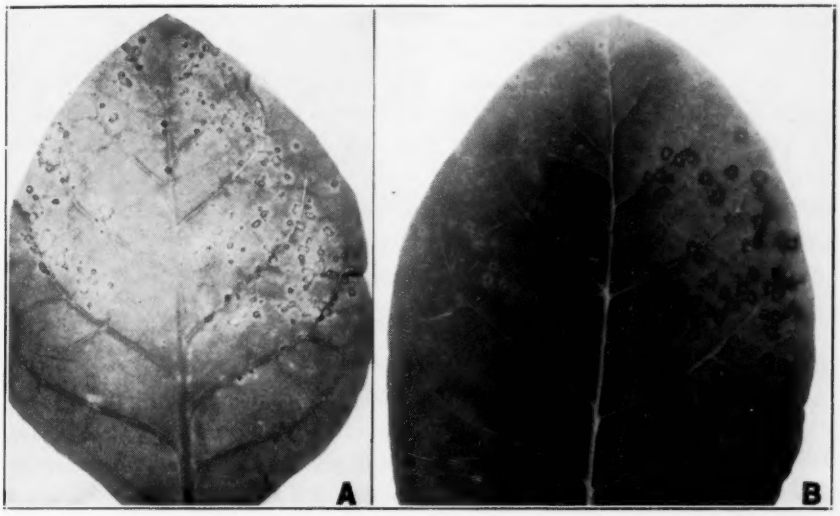

FIG. 2. A. Plantago virus collected on Ky. 16 burley (Fig. 1, C), transferred to *Plantago major* where it caused systemic infection, and then to Kelley burley (above) where it produced the necrotic reaction. Print from leaf onto direct positive paper. B. A leaf of an NN′ burley plant (F_1 Pepper N′N′ × Ky. 48–7 NN) inoculated on the left side with plantago virus and the right with tobacco mosaic virus. The N′ gene appears to be dominant over the N gene as to the plantago virus.

To test the hypothesis that Bell's field disease was caused by the plantago virus, and that the plantago virus would prove to be a necrotic spotting virus in N′ burleys, the Bell farm was visited again with the following results: Prior to 1941 the grower had placed his tobacco bed in the plowed field where the tobacco was to be grown, and had had little if any trouble from this disease. In 1941 he decided to place his beds, then and thereafter, in a grass plot surrounding his residence, which in places was found to be nearly a continuous mat of *Plantago* sp. Seven collections of plantago, 2 from the plant bed and 5 from the grass plot, proved to be infected with a virus. The plant bed had been mowed but suckers were growing from the tobacco stubs, several of which had a disease identical with the field disease. Transfers from plantago and from necrotic tobacco plants in the bed to Turkish tobacco and Ky. 16 produced identical symptoms, entirely different from the field disease but identical with the disease caused earlier by the plantago virus in these

varieties of tobacco. Transfers of the viruses from all sources were then made to plants of N′ burley varieties, Pepper and Kelley, and to an N′ dark variety, Ky. 129. On these varieties necrotic spots, of the type shown in Fig. 2, A, developed in about 48 hours on all inoculated leaves. The virus was localized in the inoculated leaves in 76.6 per cent of the 160 plants inoculated but in 24.4 per cent the virus became systemic, producing a disease entirely similar to the necrotic field disease (Fig. 1, A). It seemed evident from these results that the plantago virus, which produces a mottle disease in Turkish and Ky. 16, caused the streak-like disease of Ky. 36–12 in the Bell field.

On July 14, 1941, a virus-diseased plant, which looked as though it were affected by a strain of cucumber mosaic, was found in a field of Ky. 16 tobacco. A photograph was made (Fig. 1, C) and the virus transferred to a tobacco plant in the greenhouse. This virus gave all the reactions of the plantago virus, including systemic infection in *Plantago major;* no necrotic lesions in Scotia beans; the mottle reaction in Ky. 16, and the necrotic reaction in N′ burleys (Fig. 2, A). It may, therefore, be concluded that plantago virus in the field, as well as in the greenhouse, causes 2 distinct diseases—streak in N′ varieties and mottle in n′ varieties.[3]

DISTRIBUTION OF PLANTAGO VIRUS IN N′ TOBACCO PLANTS

When the plantago virus is inoculated to N′ burley or N′ dark tobacco plants, it may be localized in inoculated leaves or it may become systemic. When it becomes systemic, virus particles are carried to various parts of the growing points, where at least a few multiply, producing necrotic spots if the tissues are relatively old, or chlorotic spots that slowly enlarge and with age gradually become necrotic. In studies on the virus distribution in mosaic-resistant plants, it was demonstrated, through the use of bleaching strains of the virus, that virus distribution within the leaf could be determined fairly accurately by the patterns in the leaf. Patternless or green areas were virus-free, but chlorotic areas contained virus (4). Similar tests were made to determine virus distribution in diseased plants of Ky. 36–12, transplanted from the Bell farm to the greenhouse, and in plants of Pepper burley, both of which contain the N′ factor, in comparison with virus concentration in Ky. 16, which regularly becomes mottled. For this purpose 0.6-cm. disks were cut from isolated chlorotic areas and from green areas of N′ plants and from mottled areas of Ky. 16, crushed in 2 drops of M/10 disodium phosphate and rubbed with a glass spatula over as much leaf surface of an N burley plant as it would cover. Counts were made of the total spots on this area. The results are given in table 1. A high concentration of virus was found in the chlorotic and necrotic spots on the leaves of N′ plants, but green areas on the same leaves appeared to be virus-free, except in one in-

[3] In 1942 Mr. Bell used the same plant bed but substituted Ky. 16 n′n′ for Ky. 36–12 N′N′. When the bed was examined June 9, a few plants were affected by a mottle mosaic that proved to be caused by the plantago virus, and, later, many mottled plants affected with the plantago virus were found in the field.

stance. Thus, although the virus becomes systemic in N′ plants, it is limited in its distribution to a relatively few well-defined chlorotic or necrotic spots. Virus concentration in the mottled leaves of Ky. 16, inoculated with plantago virus, was high. There appeared to be no localization of the virus whatever in invaded leaves of Ky. 16.

TABLE 1.—*Virus distribution in chlorotic and necrotic areas and in green areas of N′ burley plants inoculated with the plantago virus, as indicated by the number of necrotic spots produced on N burley leaves*

Virus source	Disk from	Total disks	Total spots on N burley	Av. No. spots per disk
Ky. 36–12 Bell's field	Pepper burley, necrotic and chlorotic areas	18	3,779	210
Same	Same—green areas	17	0	0
		1	34	34
Plantago, 2 strains	Same—chlorotic and necrotic areas	48	15,756	328
Plantago, 2 strains	Same—green areas	18	0	0
Plantago, 3 strains	Plantago leaves	18	6,957	386
Plantago and tobacco, 12 strains	Ky. 16	12	5,347	445
White tobacco mosaic	Ky. 16	6	1,909	318
Yellow tobacco mosaic	Ky. 16	6	1,653	275

REACTION OF PLANTAGO VIRUS ON NN′ PLANTS

Tobacco-mosaic virus strains and the plantago virus both produce essentially the same type of necrotic spots on NN Burley 48–7, derived from Holmes' fifth backcross of Ky. 16 on *N. digluta*. On N′N′ burley the plantago and a few strains of the tobacco-mosaic virus produce necrotic spots, but most tobacco-mosaic virus strains cause only mottle. The spots on young, tender N′N′ plants are very similar to spots on NN plants, but on older plants the spots develop more slowly, are smaller, are evident first on the upper surface, rather than the lower as on NN plants, and show a much more distinct yellow halo than on NN plants. As both N and N′ factors are Mendelian dominants, and, as both affect the reaction of at least certain strains of tobacco mosaic on plants containing them, it was of interest to compare the reaction of the plantago and tobacco-mosaic viruses on heterozygous NN′ plants. Tests were first made on F_2 Burley 83–6 derived by 4 backcrosses of McMurtrey 8 N′N′ (Greenville, Tennessee, Tobacco Experiment Station) on an N burley. On field-size blooming NN′ plants of this hybrid in the greenhouse the tobacco-mosaic viruses, including those causing the necrotic reaction on N′N′ plants, all caused the usual necrotic spots. The plantago virus produced no reaction or such a weak reaction on these well-developed leaves as to be hardly noticeable. F_3 plants of 83–6, segregating for N and N′, inoculated with the plantago virus soon after transplanting to thumb pots, all developed necrotic spots and could have been classified as N. When the inoculated leaves were removed and the plants were inoculated with tobacco mosaic virus, they could be separated into those containing N and

those not containing it; because of necrosis in N plants and mottle in N′. Further tests were made on an F_1 hybrid of Pepper burley N′N′ and Ky. 48–7 NN. When these plants were growing rapidly in 4-inch pots, one half of a leaf was inoculated with tobacco mosaic and the other half with plantago virus. The large necrotic spots produced by the tobacco virus and the small spots of the plantago virus are shown in figure 2, B. Thus, in two hybrids the presence of the N′ factor has had no influence on the N reaction of the plant to the tobacco mosaic virus, but the N′ factor appears to dominate completely the reaction of the plant when inoculated with the plantago virus. In this respect the plantago virus differs from the strains of tobacco-mosaic virus that cause a necrotic reaction on N′N′ tobacco plants. On NN′ burley these tobacco-mosaic viruses cause the normal reaction of the tobacco-mosaic viruses on N plants.

TOBACCO-MOSAIC VIRUS STRAINS TRANSFERRED TO PLANTAGO

Attempts to transfer 14 distinct field strains of tobacco-mosaic virus from tobacco to *Plantago major* in the late fall resulted in 12 instances in the development of local ill-defined to well-defined necrotic spots, sometimes surrounded by concentric necrotic rings, and, in 2 instances, of no local lesions. Fourteen transfers from new leaves of inoculated *Plantago major* plants to N burley after 44 days produced only 1 necrotic spot. From the 2 inoculated plantago leaves without symptoms, 63 spots developed from one and 54 from the other. From the 12 inoculated leaves with necrotic patterns, spots ranged from 19 to 530, with an average of 311. A disk from unrubbed leaves of plantago plants inoculated with the plantago virus developed 400 spots; another disk 190 spots. Further attempts in the spring to transfer tobacco-mosaic virus strains to *Plantago major* resulted in slowly developing chlorotic spots surrounded by chlorotic rings (Fig. 1, D). In no case has it been clearly demonstrated that the tobacco-mosaic virus became systemic in *Plantago major*. It is evident that the strains of tobacco-mosaic virus tested multiply in plantago, as Holmes found, but appear to be nearly completely localized.

EFFECT OF PLANTAGO VIRUS ON TOMATO

As previously mentioned, the plantago virus studied in 1930 produced a faint mottle and some necrotic streaking in June Pink tomato. In the present studies, 7 Marglobe tomato plants, growing vigorously in a ground bench, were each inoculated, February 9, 1942, on three tip leaflets of one leaf about half way up the plant when the plants were about 27 inches tall. Other plants were inoculated in the same way with strains of tobacco mosaic that caused necrosis on N′N′ burley. The tobacco mosaics each caused systemic mosaic in tomatoes, but none of the plants inoculated with the plantago virus developed any symptoms whatever. Tests for the presence of virus in inoculated leaves and in growing points were made, March 9, 1942, on large 83–6 burley plants (N′N) before it was realized that they reacted only weakly to the plantago virus. The results were all negative from tomatoes inoculated

with plantago virus, but were positive with those inoculated with tobacco mosaics. Later, 16 Marglobe plants, 3 inches tall, were thoroughly inoculated on two leaves with one of 3 strains of plantago virus. All developed systemic mottling and 12 developed some streak. In April 3-inch June Pink plants, just becoming established in 4-inch pots, were inoculated. Three lots of 4 plants each were each inoculated with a different collection of the plantago virus. Two plants of each lot were inoculated on all leaflets of the first 2 leaves and the other 2 were inoculated on the tip half of 3 leaflets of 1 leaf. No local lesions developed, but soon systemic mottling occurred in the heavily inoculated plants, accompanied by mild necrotic streaking of the stalks and midveins and necrotic spots or rings in invaded leaves. As growth continued, symptoms were limited to isolated chlorotic spots in the new leaves that caused some distortion and prominent mottling. Of the lightly inoculated plants, only 2 became systemically invaded 3 weeks or more after inoculation. When the above test was repeated on 8-inch June Pink tomato plants in 6-inch pots, infection developed, after 35 days, only in the heavily inoculated plants.

Later, 4 Marglobe plants 20 to 27 inches tall were inoculated on 3 leaflet tips of 1 leaf or on all leaflets of 3 leaves about two-thirds up the plant. None of these plants developed systemic infection. In tests of the leaf tissue, using 3 0.6-cm. disks from actually inoculated tissue of each plant, only 5 of 11 trials produced necrotic spots on a necrotic-spotting NN burley.

The results of these tests suggest that tomato is resistant to the plantago virus, but, if young, vigorous plants are heavily inoculated, the virus will become systemic. As the leaves age they appear to become more resistant to both local and long-distance spread of the virus. The resistance of the tomato to the plantago virus is not very different from that of mosaic-resistant Ambalema burley hybrid plants to the tobacco-mosaic virus.

DISCUSSION

The widespread distribution of a virus in plantago, which is so closely similar to the tobacco-mosaic virus that Holmes claimed "it was a strain of tobacco-mosaic virus (*Marmor tabaci* H.)," is of interest from the standpoint of the origin of a virus of a cultivated crop, such as tobacco. There seems to be no question that the plantago virus and the numerous field tobacco-mosaic virus strains are closely related. But to claim that plantago virus is a strain of the tobacco-mosaic virus implies that the changes in the virus have been from the tobacco-mosaic virus to the plantago virus, rather than in the opposite direction. On the contrary, it is not unreasonable to assume that the plantago virus antedates the allopolyploid species *Nicotiana tabacum* and cultivation of tobacco by the Indians, and, therefore, antedates the tobacco-mosaic virus itself. While there are species of *Nicotiana* that are susceptible to tobacco mosaic, it is doubtful whether in nature the virus maintains itself in these annual species. But there would be no difficulty in the virus maintaining itself indefinitely in a perennial species such as *Plan-*

tago major and attaining a very wide geographic distribution in the absence of any effective insect vector. Any mechanical means of distribution, such as the hoofs of animals tramping over diseased and then healthy plants, should be sufficient to bring about widespread distribution of a virus that withstands drying and is as easily transmitted as the plantago virus.

With the development of tobacco and its cultivation by the aborigines, the virus could readily have been transferred from plantago to tobacco, as illustrated on the Bell farm. If the variety of tobacco happened to be of the n′n′ genetic make-up, then a mottle disease would result which could be passed on indefinitely in cultivated tobacco grown by men who handled tobacco from the previous infected crop. Holmes has shown that there is a slight tendency for the plantago virus to develop substrains and in the present study a collection made in Connecticut developed the burning type of necrosis much more slowly in Ky. 16 than the local strains. Therefore it may be assumed that the virus is variable but confined, in its variability, to the limitations placed upon it by the genus *Plantago*.

Man, in handling tobacco plants with fingers contaminated by viruliferous dry tobacco, gives a means by which mutant strains can be isolated and then perpetuated. Blood (1) has demonstrated the ability of the tobacco mosaic virus to mutate sufficiently so that it could cause mosaic mottle in datura plants that previously showed the necrotic reaction. When the mutant virus was transferred to other datura plants, it no longer caused necrosis but caused a mottle disease. Thus by mutation, strains of the plantago virus might arise capable of causing mottle in N′ plants, and gradually the numerous field strains of the common tobacco mosaic could have been evolved. On the other hand, considering the high degree of resistance of plantago to systemic infection by the tobacco-mosaic virus, it is unlikely that the plantago virus originated from the tobacco-mosaic virus and was then distributed over its wide range in a relatively pure form.

In studies of the common field strains of the tobacco-mosaic virus for the past 20 years, the writers have failed to find any evidence for a type strain, but have found a nearly limitless series of strains, such as would be expected from a mutating species given opportunity to perpetuate its mutant strains. Each of the strains appears limited in its distribution, but any one of them may be maintained as a laboratory strain. Holmes has named the plantago virus *Marmor tabaci* var. *plantaginis*, thus making it coordinate with the strains of the tobacco-mosaic virus that he has recognized with Latin varietal designations. Obviously, it is not coordinate with them because it is a virus with well-defined characters, is well established in nature over a wide geographic range, and is found on a distinct group of plants, whereas his tobacco mosaic varieties are ill-defined, are not established in nature, and are obviously mutant strains of a well-recognized virus. This would seem sufficient, according to the criteria proposed by Valleau (7, p. 821), to give the plantago virus a varietal designation in contrast with the numerous mutant strains of the tobacco-mosaic virus, which should be given only laboratory designations.

Even though the plantago virus proves to be the stem from which the tobacco-mosaic viruses have arisen, it would seem necessary, because of priority, to designate it a variety of the species *tabaci*. According to the system proposed by Valleau, the virus would be called *Musivum tabaci* var. *plantaginis* new comb.

SUMMARY

The plantago virus is common in *Plantago* sp. in the vicinity of Lexington, Kentucky. An outbreak of a necrotic virus disease of burley tobacco was found to be caused by the plantago virus in a variety that responded with the necrotic reaction. In some other varieties, the same virus causes a mottle disease. Both types of reaction were found in tobacco plants naturally infected.

In tobacco carrying the N factor from *Nicotiana glutinosa*, the plantago virus and the tobacco-mosaic virus cause similar necrotic reactions, but, in tobacco carrying the N′, as well as the N, factor, the plantago virus produces few minute necrotic spots, while the tobacco mosaic virus causes the usual necrotic reaction.

The suggestion is made that the plantago virus may have antedated the origin of *N. tabacum* and that the numerous strains of the tobacco-mosaic virus may have derived from the plantago virus by mutation.

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THE ZONATE LEAF SPOT, A NEW DISEASE OF SORGHUM

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In the fall of 1940, a leaf spot was noted on the leaves of several varieties of sweet sorghum (*Sorghum vulgare* Pers.) growing on the experiment station grounds of Louisiana State University at Baton Rouge. Entire leaves of most of the plants were covered with large and somewhat zonate spots, and in these spots were numerous black sclerotia-like bodies.

Diseased leaves were placed in moist chambers, while bits of others were surface-sterilized and planted in bean-pod agar. A fungus with filiform conidia, borne in a slimy matrix, was obtained so consistently that it seemed advisable to make inoculation tests. Results of the inoculations indicated that the fungus was definitely pathogenic.

Attempts to identify the fungus were, from the beginning, unsatisfactory. It resembled in many ways a fungus that Miura (5) described from Manchuria in 1921 under the name *Ramulispora andropogonis* Miura. Later, in 1932, following the ideas of Bubak (4), the name was changed to *Titaeospora andropogonis* (Miura) by Tai (6). The genus *Titaeospora*, however, is characterized by the presence of branched conidia. Although the conidia of the sorghum fungus were definitely not branched, there was a possibility that the branching of the spores was not a fixed character and occurred only under certain conditions. Because of the apparent similarity of other characters, the sorghum fungus was then tentatively considered the same as the Manchurian fungus (1, 2). This, however, was later proved incorrect (3). In the summer of 1941, a fungus, definitely determined as *Titaeospora andropogonis*, was collected on Johnson grass (*Sorghum halepense* (L.) Pers.). This fungus bore the typical branched spores, as described for *Titaeospora*, and was shown to be different from the sorghum fungus. It seemed clear, then, that the sorghum fungus was not only a new species but also did not fit satisfactorily in any of the commonly known genera of the *Fungi Imperfecti*.

In the fall of 1941, a culture of the fungus, stained paraffin sections, and fresh mounts of the fruiting body on the host were sent to C. L. Shear for identification. Shear's reply was as follows: "This is a very interesting organism, and I have spent considerable time in attempting to identify it but have been unable to find any description of either genus or species which agrees with it. It suggests a *Cercospora* somewhat but differs in having slimy spore masses, and very short conidiophores and a *Sporodochium*-like base unlike most *Cercospora* species. It seems to be nearer the *Tuberculariaceae*. We are tentatively calling it *Gloeocercospora heterospora* n. gen. & sp. I do not feel quite sure at present whether all of the various forms, shapes, and sizes of conidia are mere variations of one form or whether there are two forms, one shorter and slightly thicker than the other. The sclerotia formed in culture are also an interesting feature of the fungus."

Based on a study of conidia from single-spore cultures and from the host, it is evident that the differences in the conidia are only variations of one form. Since the term "heterospora" suggests more than one distinct type of conidium, and since the organism occurs on species of the genus *Sorghum*, the name *Gloeocercospora sorghi* is now being proposed for the fungus.

SYMPTOMS OF THE DISEASE

The leaf spots produced by *Gloeocercospora*, when well developed, can usually be distinguished from other sorghum leaf spots. Initial lesions ap-

FIG. 1. Leaf spots produced by *Gloeocercospora sorghi* on leaves of sweet sorghum. A. Spots produced by natural infection. B. Spots produced by inoculation with pure culture.

pear as small, reddish or brownish, water-soaked spots that sometimes have a narrow, pale-green halo. Later, as the spots enlarge, they become a dark-red, except in certain varieties where they are light-brown, and become somewhat elongate and parallel to the veins. They finally form (possibly by coalescence) large, semi-circular, or irregular lesions several centimeters

in diameter (Fig. 1). A smaller lesion usually has a light-brown center, surrounded by a light- to dark-red border; but, frequently, in larger lesions there may be an alternation of dark and light zones. These leaf lesions may occur along the margins or towards the midrib, or they may cover the entire leaf when infection is heavy. Often the younger red lesions are so numerous as to form red irregular blotches. The zonate spots have been observed as early as the first part of June. Because of the characteristic type of spotting, the name "zonate leaf spot" is suggested for the disease.

A few weeks after infection occurs, minute spherical to lenticular sclerotia appear in the necrotic areas of infected leaves and sheaths. Leaves and sheaths are the only parts of the plant on which symptoms have been observed. The fungus, however, has been isolated from surface-sterilized seeds and glumes, which indicates that these structures also become infected.

HOST RANGE AND DISTRIBUTION

The fungus was widely distributed in 1941 in southern Louisiana, being found on sorghum, Johnson grass, and Sudan grass (*Sorghum vulgare*, var. *sudanense* (Piper) Hitchcock). During the summer of 1941, typical zonate lesions were noted at Meeker, Louisiana, on one variety of sugarcane, C.P. 33/243. Leaves showing these lesions, when placed in a moist chamber, produced within a few days, conidia of *Gloeocercospora*. In 1942, the fungus was also collected on corn (*Zea mays* L.) at Baton Rouge.

The distribution of the fungus outside of Louisiana is indefinite. It was collected in 1941 on Johnson grass and sorghum at Poplarville, Mississippi. That it may have a wider distribution is indicated by a culture kindly sent by C. L. Lefebvre. This culture was isolated from Sudan grass from Arlington Farm, Virginia, in 1939. This isolate, except for slight cultural differences, proved to be quite similar to cultures isolated from sorghum in Louisiana. During 1942, specimens of the fungus on sorghum were received from G. F. Weber, Gainesville, Florida, and specimens of apparently the same fungus on seedlings of *Agrostis* were received from C. C. Wernham, State College, Pennsylvania.

THE FUNGUS

Gloeocercospora sporulates on the host under field conditions but the large, well-defined fruiting bodies usually are inconspicuous. However, when infected material is placed in a moist chamber for 24 to 48 hours, an abundance of fruiting bodies appear in and surrounding necrotic areas.

From a study of prepared sections, it appears that the fruiting body is a sporodochium, and that this structure is found on the surface of the leaf above a stomate and arises from hyphae emerging from the stomate (Fig. 2, A). These hyphae branch and a sporodochial column is formed that, at maturity, is more or less definitely stalked. The branching conidiophores produce a bouquet-like structure (Fig. 2, B). The sporodochium apparently never originates within the tissue of the leaf and becomes

erumpent. There is no stromatic base above the leaf surface or in the substomatal cavity. The sporodochia are salmon colored and are easily visible to the naked eye. They occur either in dense clusters or sparsely in and around necrotic areas.

The conidiophores are so densely clustered that it is difficult to determine their length and width. In general, they appear to be short (5 to 10 μ), hyaline, and either simple or branched.

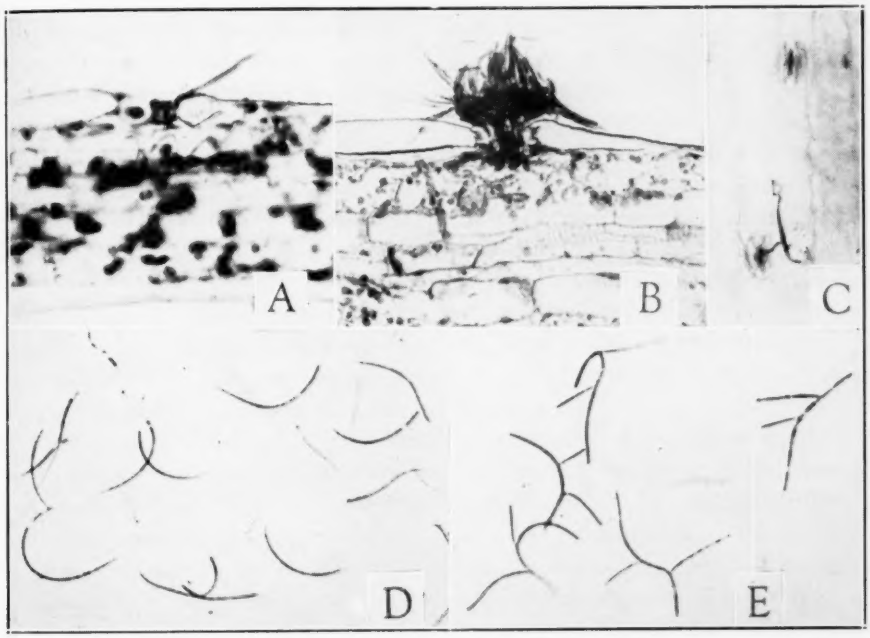

FIG. 2. A–D. *Gloeocercospora sorghi*. A. Young sporodochium, × 500. B. Mature sporodochium, × 320. C. Germ tube penetrating stomate, × 240. D. Spores, stained with cotton blue, × 320. E. Spores of *Titaeospora andropogonis*, stained with cotton blue, × 320.

The conidia (Fig. 2, D) are borne in a pinkish to salmon slimy matrix. They are either straight or curved, tapering somewhat from the base to the apex, few- to many-septate, hyaline, and elongate to filiform. The length varies from 20 to 195 μ and at the widest place they are slightly over 3 μ in width.

The black sclerotia develop within the tissues of the older leaf lesions. They occur at definite intervals and in lines parallel to the veins, which suggests that they form under the stomates. The host tissue, however, is so badly disintegrated by the time the sclerotia are fully developed that it is difficult to determine with certainty their relation to the stomates. In section, the sclerotia are round to elliptic. Each sclerotium has a central portion composed of pseudoparenchymatous tissue that is surrounded by a hard layer composed of thick-walled cells.

Gloeocercospora grows rapidly on ordinary culture media, often filling a

Petri dish in less than 10 days. It sporulates abundantly, especially on oatmeal agar. The optimum temperature for growth is in the neighborhood of 28° to 30° C.

The conidiophores in culture are hyaline, simple or branched, short, and septate. They have been observed to arise pleurogenously from hyphae as rather densely clustered short branches. Later, these clusters of conidiophores may become so dense as to form bouquet-like aggregates, resembling the sporodochia that are formed on leaves.

In culture the conidia, which develop in pink, bead-like, slimy masses, do not differ materially from those on leaf lesions. These masses often coalesce to form larger ones. Under optimum conditions, sporulation has occurred in 4 days. A conidium is attached somewhat on the side of the slightly swollen apex of the conidiophore (or its branch) and by its broadest end.

Sclerotia resembling closely those in the host develop on culture media.

The conidia germinate readily in water or culture media generally in less than 5 hours. Frequently, the different cells of the conidium produce germ tubes, as many as 6 having been observed coming from one spore.

In inoculation experiments it has been determined that the germ tubes of the germinating conidia enter the host through stomates. In the tests, potted sorghum plants in the greenhouse, plants growing in the field, and detached leaves in moist chambers were used. In one of the tests, the plants were kept under bell jars for 48 hours after being inoculated by spraying the leaves with a spore suspension. Beginning 6 hours after inoculation, material was killed and fixed in a 50 per cent solution of acetic acid in alcohol and cleared in a saturated solution of chloral hydrate. When ready for examination, leaf sections were stained with a dilute solution of cotton blue in lacto-phenol. The fungus took the stain, while the leaf tissue remained clear. Material killed 24 hours after inoculation was found most suitable for study.

In all the material examined, no appressoria were found, nor was there any evidence of penetration of the epidermis. Entrance of the germ tubes through stomates was observed in many instances (Fig. 2, C). In each one noted, penetration was effected before branching of the germ tube had occurred. Often, the germ tubes appeared slightly swollen at the point of contact with the stomatal aperture. It was noted also that germ tubes would frequently grow over and beyond nearby stomates without entering them.

In most of the experiments, brownish spots began to appear on the leaves about 24 hours after inoculation, apparently at about the time the germ tubes were entering the stomates.

CLASSIFICATION OF THE FUNGUS

In the attempts to identify and classify the fungus, it has been necessary to compare it with organisms found in such genera as *Cylindrosporium*, *Titaeospora*, *Cercospora*, and *Cercosporella*. There is no evidence, however, that would warrant the placing of the sorghum fungus in any of these genera.

The fruiting body of *Gloeocerspora* is a sporodochium-like structure and, on this account, it is believed that the fungus should be placed in the *Tuberculareaceae*.

The genus *Cylindrosporium* is ordinarily placed in the *Melanconiales*, a group characterized by the presence of an acervulus, a structure that is innate in the matrix and finally becomes erumpent. A fruiting structure like that found in *Gloeocercospora*, which originates between the guard cells or slightly below and emerges through the stomate, cannot be considered an acervulus. For this reason, *Gloeocercospora* should not be placed in the genus *Cylindrosporium* or in the order *Melanconiales*. As the spore characters of *Gloeocercospora* resemble those of some of the species at present classified in the genus *Cylindrosporium*, it would be interesting to determine if some of the latter fungi may not have fruiting bodies similar to *Gloeocercospora*.

The fungus, *Titaeospora andropogonis*, is also at present placed in the *Melanconiales*. The fruiting body, however, is not a typical acervulus but a sporodochium-like structure, and the fungus probably should be placed in some other order. The stroma-like base of the sporodochium underneath the stomate and the branching of the spores (Fig. 2, E) are characters of *Titaeospora andropogonis*, which definitely distinguish it from *Gloeocercospora*.

Gloeocercospora differs from *Cercospora* and *Cercosporella* in that its fruiting body is definitely a sporodochium and the conidia are borne in a slimy matrix on short conidiophores.

TECHNICAL DESCRIPTION

Gloeocercospora gen. nov.

Vegetative hyphae septate; fruiting body a sporodochium formed on the surface of the host above the stomatal opening and arising from hyphae that emerge through the stomate; conidiophores hyaline, septate, simple or branched, short; conidia hyaline, elongate to filiform, of variable length, the longer ones tapering, acrogenous, 1-multiseptate, straight or curved, borne in a slimy matrix.

Gloeocercospora sorghi sp. nov.

Vegetative hyphae septate, hyaline, branching; sporodochium between guard cells and above stomatal aperture; conidiophores hyaline, septate, simple or branched, short, 5 to 10 μ; conidia hyaline, elongate to filiform, of variable length, the longer ones tapering 20–195 × 1.4–3.2 μ, average 82.5 × 2.4 μ, borne in a slimy matrix, salmon-color in mass; sclerotia 0.1–0.2 mm. in diameter, lenticular to spherical, black, occurring inside the necrotic tissue of the host, abundant. Parasitic on leaves of *Sorghum halepense* (L.) Pers., varieties of *S. vulgare* Pers., and other grasses.

Type locality: Baton Rouge, Louisiana, U. S. A.

Type material including leaves, slides, and dried agar cultures of the fungus has been deposited in the herbarium of the Department of Botany, Louisiana State University and the mycological collections of the Bureau of Plant Industry, Washington, D. C.

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ANOTHER HYPHOMYCETOUS FUNGUS PARASITIC ON PYTHIUM OOSPORES

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In an earlier paper (3) 2 hyphomycetous fungi, *Trinacrium subtile* Riess and *Dactylella spermatophaga* Drechsl., were reported as having been found subsisting parasitically on mature oospores formed by root-rotting oomycetes in isolation plate cultures prepared by planting sizable fragments of diseased vegetable tissue on maize-meal agar. From their morphological features and developmental traits these 2 fungi could be recognized as closely akin to the series of mucedinaceous forms most familiarly exemplified in *Arthrobotrys oligospora* Fres., the larger number of which, under natural conditions, habitually secure their nourishment by capturing nematodes, amoebae, and testaceous rhizopods.

More recently a third fungus of like biological habit and presumably also of like taxonomic kinship came to light in an old Petri-plate culture of *Pythium graminicolum* Subr., following the addition of a small quantity of leaf mold taken from woods near Haugen, Wis., in September 1939. Throughout a tract of approximately 500 square millimeters bordering the deposit of forest refuse, many thousands of oospores were found reduced to membranous remains. Day after day other oospores could be observed undergoing destruction along the margin of the slowly enlarging tract. The units of sexual apparatus about to be attacked arrested attention by the development of a distended crook-necked structure whose broadly rounded apex was closely appressed to the oogonial membrane; so that an appearance curiously suggestive of fertilization was presented, though true fertilization had everywhere been accomplished fully 2 months earlier. The crook-necked structure, while closely resembling the antheridia of various species of *Pythium*, including *P. graminicolum*, soon revealed its proper character by functioning as an appressorium in thrusting a process, often 1.5 to 2 μ wide, through the oogonial membrane and into the oospore wall. Within this wall the intruded process would sometimes gradually expand for about 2 hours and thus form a conspicuous bulbous enlargement 8 μ or 9 μ in transverse diameter (Fig. 1, A–D) before breaking into the chamber of the spore (Fig. 1, E) and extending throughout the protoplast a rather massive, somewhat branched haustorium (Fig. 1, F, G). Usually perforation of the oospore wall was accomplished with less pronounced inflation, the invading process, in most instances, becoming distended to a width of perhaps 4 μ or 5 μ in passing through the thick envelope (Fig. 1, H, I).

The protoplasmic content of the oospore usually revealed no change in its normal unitary organization (Fig. 1, A) until the invading fungus had almost penetrated the enveloping wall. At that stage the reserve globule often lost its smoothly circular contour, while, simultaneously, the refringent

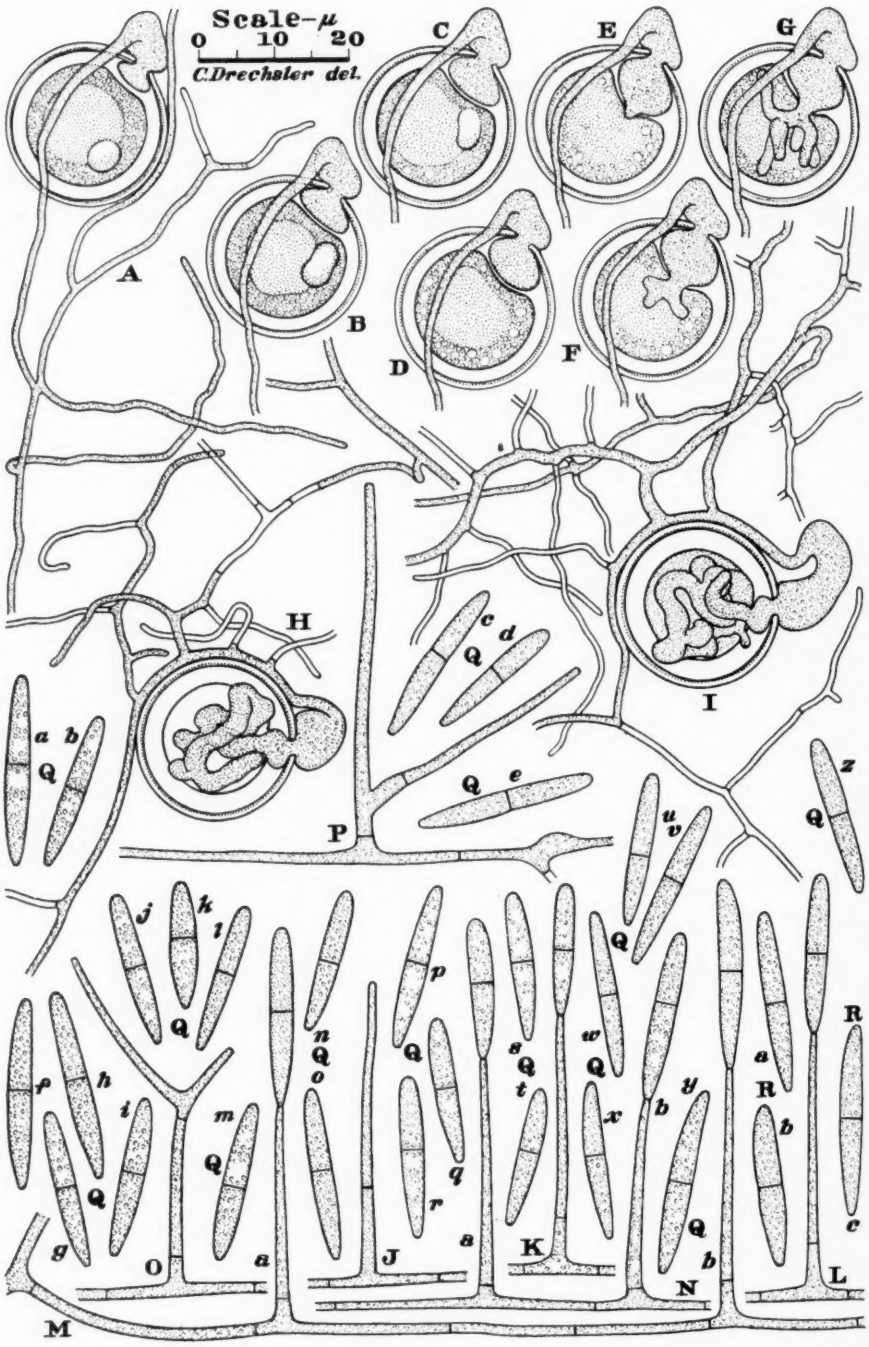

FIG. 1. *Trichothecium arrhenopum* drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A–G. Successive stages in the penetration and invasion of an oospore of *Pythium graminicolum* observed during 2½ hours. H, I. Portions of mycelium, each with an appressorium from which an oospore of *P. graminicolum* has been invaded. J–N. Portions of prostrate hyphae bearing one or two simple erect conidiophores. O, P. Branched conidiophores. Q, a–z; R, a–c. Random assortment of conidia, showing variations in size and shape.

body assumed an elongated (Fig. 1, B) or irregular outline. Soon afterwards the parietal granular layer became interspersed with numerous small vacuoles, and merged more and more with the misshapen reserve globule (Fig. 1, C, D). By the time the invading fungus had irrupted into the chamber of the oospore the refringent body often could no longer be clearly distinguished (Fig. 1, E–G). Gradually the disorganized mass of protoplasm became less dense in texture as the substances composing it were absorbed by the haustorium. The oospore wall became noticeably swollen and evidently underwent some softening (Fig. 1, H, I), though it was not apparent that the softened materials were assimilated. Eventually, after the granular residues of the host had disappeared, the protoplasmic contents of the haustorium itself were withdrawn into the external mycelium of the parasite.

This mycelium consisted of delicate hyphae giving off branches rather promiscuously in all directions (Fig. 1, A, H, I). Many of the finer ramifications were appreciably narrower than 1 μ, while the axial filaments from which they arose often did not exceed 1.5 μ in width. The delicate vegetative habit appeared to represent an adaptation whereby the parasite is enabled, with obvious economy, to seek out oospores in scattered positions. When an oogonium containing an oospore was encountered, the successful exploratory branch enveloped the oogonial membrane very tightly about half way around its circumference (Fig. 1, H, I) before giving rise terminally to the massive crook-necked appressorium already described. Unsuccessful exploratory branches, on the other hand, were soon evacuated through retraction of their protoplasm into the parent filaments. Septa could not be recognized within any portion of the exploratory mycelium that remained full of protoplasm, though partitions were readily visible within empty portions of hyphae, as well as at the boundaries of living portions. Perhaps intercellular septa, as distinguished from retaining walls, were omitted here in order to promote freer movement of protoplasm, and thus to facilitate the ready production and extension of exploratory branches. However, it is also possible that intercellular septa were present, but, owing to the small dimensions of the filaments, were indiscernible to ordinary microscopical inspection. The apparent continuity of its living portions made the submerged parasitic mycelium resemble in some degree the mycelium of a delicate phycomycete.

That the parasite is, nevertheless, not a phycomycete was evident from the indubitably septate condition of some coarser, more straightforward, and only sparingly branched hyphae formed on or near the surface of the agar medium (Fig. 1, J–P). These hyphae were concerned more directly with the asexual reproduction of the fungus than with its vegetative development, since they gave rise at moderate intervals to erect, slender, colorless, frequently uniseptate, aerial conidiophores (Fig. 1, J–L; M, a, b; N, a, b), which usually concluded their development by bearing a single colorless elongated conidium. Sometimes, to be sure, a conidiophore, after declining to the substratum, extended its usefulness by sending up a secondary

conidiophore as a lateral branch (Fig. 1, O, P); the secondary conidiophore, after bearing its single conidium, then occasionally falling over in turn and giving rise to a tertiary fertile branch. With respect to such protracted development, the fungus showed similarity not only to the oospore-destroying *Dactylella spermatophaga*, but also to many of the more robust members of the predaceous series of hyphomycetes subsisting habitually through capture of animals.

The conidia (Fig. 1, Q, a–z; R, a–e), whether produced on simple conidiophores or on branches arising therefrom, were only about two-thirds as wide and less than half as long as those of *Dactylella spermatophaga*, from which they differed further in being divided regularly by a single cross wall rather than by 3 cross walls. Their meager septation, blunt ends, and lack of pronounced curvature, removed them from any close resemblance to the macroconidia of *Fusarium*, though some general similarity to the much less distinctive microconidia produced by members of that genus was evident. Taxonomic disposition of the fungus is, therefore, not disturbed by such perplexities as are attached to the curiously ambiguous conidial morphology of *D. spermatophaga*.

The character of its asexual reproductive apparatus clearly refers the fungus to *Trichothecium* Link in the more restricted sense in which this genus was adopted by Saccardo (8, v. 4, p. 178). *Trichothecium*, throughout the *Sylloge Fungorum*, is held distinct from *Cephalothecium* Corda,—the former being made to include species that bear uniseptate, hyaline or light-colored conidia singly on simple erect conidiophores, while the latter is reserved for species bearing such conidia plurally in more or less capitate arrangement on conidiophores likewise erect, simple and septate. The distinction thus drawn has fallen somewhat into disfavor from a belief that *T. roseum* Link and *C. roseum* Corda, on which, respectively, the 2 genera would seem to have been founded, and by which, certainly, they have long been most familiarly exemplified, represent one and the same fungus. The fungus that presumably is in question here, best known to students of plant diseases from its causal connection with pink rot of apples, may, indeed, be found bearing solitary spores on conidiophores just at the beginning of their reproductiveness, whereas its older conidiophores are commonly found supporting a number of spores in a contorted spicate cluster having the general appearance of a head. Owing to the formation of the conidia one after another, in basipetal order, the clustered condition is necessarily preceded for a brief period in the development of the individual conidiophore by a condition that could be interpreted as expressive of a solitary sporulating habit; though the usual prevalence of a clustered arrangement wherever the fungus is present in quantity should remove virtually all occasion for such error.

The earlier definitions of *Trichothecium* were flagrantly wanting in morphological detail. Link failed to specify any particular manner of spore formation both in his first (5, p. 18) and in his second diagnosis (6 p. 28)

of the genus. Nor is any particular manner of spore formation mentioned in his diagnosis of *T. roseum* (6, p. 28); and the single relevant figure given by him (5, Tab. 1, 27) merely shows detached uniseptate spores scattered promiscuously among a number of septate hyphae. The usage that in early times grew up with regard to the species would seem to have been based more on considerations of color, habitat, and distribution than on considerations of morphology. Confusion of more than ordinary seriousness arose. As late as 1912 Grove (4) held that *T. roseum*, *T. candidum* Wallr., *T. obovatum* (Berk.) Sacc., *C. roseum*, *C. candidum* Bon., *Arthrobotrys superba* Corda, *A. oligospora*, and *A. rosea* Mass. might represent varying states of one species.

The citation of *Arthrobotrys superba* and of *A. oligospora*, with which *A. rosea* appears identical, among the dubious species of *Trichothecium* and *Cephalothecium* was without much justification, since the distinction between the truly capitate sporulation of *A. oligospora* and the successive, basipetal production of conidia in the pink-rot fungus had been repeatedly pointed out during the preceding half-century. This difference in manner of cluster development seems, indeed, of far greater taxonomic significance than the difference between production of single capitate clusters and successive capitate clusters; wherefore, some years ago, in describing as new 2 nematode-capturing species that give rise only to single capitate clusters, I ventured to assign them under the names *A. musiformis* and *A. dactyloides*, to *Arthrobotrys* (3) rather than to *Cephalothecium*, as would have been required in strict accordance with Saccardian usage. Through such extension of *Arthrobotrys* at the expense of *Cephalothecium*, the latter genus, of questionable validity because of its erection subsequent to *Trichothecium*, is conveniently excluded from the predaceous series of hyphomycetes. Of the 7 species compiled under *Cephalothecium* in the *Sylloge Fungorum*, *C. macrosporum* Speg. with fertile hyphae "apice minute abrupteque nodulosо-sterigmatophoris" (8, v. 10, p. 549), and *C. microsporum* Eichelb. with conidiophores "apice inflatis et minute verruculosis" (8, v. 22, p. 1305) might perhaps belong in the predaceous series. On the other hand, although Zopf's figure of *C. roseum* (9, p. 309, fig. 26, IV) shows uniseptate conidia borne in pronouncedly capitate arrangement on an erect conidiophore, the large, subspherical, terminal, *Oedocephalum*-like enlargement to which the conidia are attached seems scarcely less alien to *Arthrobotrys* than to *C. roseum* as originally described and figured by Corda (1, Tab. X, fig. 62).

The hyphomycetous forms intimately akin to *Arthrobotrys oligospora*, but producing their uniseptate conidia singly, offer no prominent morphological feature whereby they can be separated readily from the forms that while likewise producing solitary uniseptate conidia on erect sporophores are related to *Trichothecium roseum*. Under natural conditions of development a heavy, somewhat crustose, and rather bright-colored turf of sporiferous hyphae is likely to denote affinity with the pink-rot fungus, while scant development of colorless or only faintly tinged sporiferous hyphae would

more probably betoken affinity in the predaceous series,—the abundant display of conidial apparatus in the former instance being referable to direct utilization of the substratum as food supply, whereas the meager display in the latter instance derives from utilization only of minute animals or fungal structures associated with the substratum. Because of its very delicate aerial development *T. griseum* Speg. (8, v. 4, p. 180), for example, may be suspected of belonging in the predaceous series. The similarly very delicate *T. inaequale* (8, v. 18, p. 539) described by Massee and Salmon (7) from horse dung and rabbit dung in England, almost certainly is a member of this series, and very probably, in view of its close resemblance to the nematode-capturing fungus I described as *T. polybrochum* (2), habitually subsists on eelworms. As the fungus found parasitizing oospores of *Pythium graminicolum* gives rise to conidial apparatus in quantity so small as to be wholly invisible to the naked eye, its presence would ordinarily escape detection on opaque natural substrata. At all events, it cannot be identified with any of the 18 species compiled under *Trichothecium* in the *Sylloge Fungorum*, and has apparently not been described hitherto. It is, accordingly, presented as a new species under a specific name meaning "masculine-looking," which is intended to be descriptive of its antheridium-like appressoria.

Trichothecium arrhenopum sp. nov.

Mycelium bonam partem valde ramosum, hyphis incoloratis, certe ex parte septatis, plerumque 0.6–2 μ crassis, in oogonium Pythii maturum incasis id partim arte circumplectentibus denique appressorium ei late applicantibus; appressorio plerumque 5–8 μ crasso, 8–18 μ longo, ad instar antheridii curvato, membranas oogonii oosporaeque perforante denique ramos assumentes 1.5–3 μ crassos intrudente. Hyphae fertiles erectae, incoloratae, pauce septatae, plerumque 25–50 μ altae, basi 1.7–2.5 μ crassae, apice 0.7–1.5 μ crassae; conidiis solitariis, incoloratis, clavulatis vel elongato-ellipticis, basi obtusis, sursum rotundatis, medio septatis, plerumque 17–25 μ (saepe circa 21.4 μ) longis, 2.6–3.7 μ (saepe circa 3.1 μ) crassis.

Habitat in humo silvestri prope Haugen, Wisconsin.

Mycelium in large part abundantly branched, and in part, at least, septate; the vegetative hyphae colorless, mostly 0.6 to 2 μ wide, on encountering a mature *Pythium* oogonium enveloping it very closely along approximately one-half of its circumference and then producing an appressorium in broad contact with it; appressorium mostly 5 to 8 μ wide, 8 to 18 μ long, curved or crook-necked after the manner of some antheridia, after penetrating the walls of both oogonium and oospore intruding a haustorium with branches 1.5 to 3 μ wide to appropriate the protoplasmic contents. Conidiophores erect, colorless, usually with a single septum near the base, measuring mostly 25 to 50 μ (average 34.4 μ) in height, 1.7 to 2.5 μ (average 2.2 μ) in basal width, and 0.7 to 1.5 μ (average 0.9 μ) in apical width. Conidia solitary, colorless, somewhat clavate or of elongate-elliptical outline, blunt at the base, rounded at the tip, mostly 17 to 25 μ (average 21.4 μ) long, 2.6 to 3.7 μ (average 3.1 μ) wide, divided by a median septum, the position of which is sometimes marked by a slight constriction.

Occurring in leaf mold near Haugen, Wis.

SUMMARY

A delicate mucedinaceous fungus from Wisconsin leaf mold was found energetically parasitizing oospores of *Pythium graminicolum* in an agar culture. It is described as a new species under the name *Trichothecium arrhenopum*. Like *Trinacrium subtile* and *Dactytella spermatophaga*, which have previously been made known as oospore parasites, it seems closely related to the series of predaceous hyphomycetes exemplified in *Arthrobotrys*

oligospora rather than to the familiar *T. roseum*. It penetrates the oogonial envelope and the oospore wall by means of a massive crook-necked appressorium having a curious resemblance to the antheridia of many species of *Pythium*.

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SEEDLING BLIGHT AND ROOT ROT OF GRASSES IN MINNESOTA[1]

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There have been numerous complaints of failure to obtain stands of various species of grasses, even when seeds are sown in apparently favorable seed beds. Furthermore, 1-year-old plants have been observed injured by root rot in nurseries, the damage being especially severe on the Forage strain of crested wheatgrass.

Because of the difficulties encountered in obtaining and maintaining stands of grasses, work was begun in the fall of 1939 to determine the cause. Seed of the following grasses were surface sterilized for 3 minutes in mercuric chloride and plated out on acid potato-dextrose agar: *Bouteloua gracilis* (H. B. K.) Lag., *B. curtipendula* (Michx.) Torr., *Agropyron cristatum* (L.) Beauv., *A. pauciflorum* (Schwein.) Hitchc., *A. smithii* Rydb., *Andropogon furcatus* Muhl., *A. scoparius* Michx., *Bromus inermis* Leyss., *Buchloë dactyloides* (Nutt.) Engelm., *Elymus canadensis* L., *Panicum virgatum* L., *Poa ampla* Merr., and *Sorghastrum nutans* (L.) Nash. Many fungi, including *Helminthosporium*, *Alternaria*, *Fusarium*, *Cladosporium*, and *Penicillium* spp., were obtained from a total of 1,500 seeds. Bacteria, also, were commonly isolated.

Surface-sterilized and nonsterilized seed of the above species of grasses also were planted in steamed and nonsteamed sand. No significant differences were observed in germination capacity, time of germination, or in seedling vigor. Therefore, work with the organisms isolated from seed was discontinued and attempts were made to isolate organisms from the roots of grass seedlings grown in soil from different sources.

Seed of *Agropyron smithii*, *Andropogon scoparius*, *Bouteloua gracilis*, *B. curtipendula*, *Bromus inermis*, *Poa ampla*, and *Sorghastrum nutans* were planted in the greenhouse in soils abtained from Brookings, South Dakota, and Waseca and St. Paul, Minnesota. Organisms were isolated from the roots of the resulting plants by surface-sterilizing tissue with mercuric chloride and then plating out on acid potato-dextrose agar. The isolates obtained were then tested for pathogenicity in a preliminary way by placing them in a plate of water agar in which seedlings of bromegrass or crested wheatgrass were growing. If the roots of the seedling turned brown or died, the isolate was considered pathogenic. Table 1 lists the organisms isolated from roots in each of the soils and the pathogenic effects of each.

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TABLE 1.—*Soil borne organisms isolated from the roots of grass seedlings grown in soils from three different sources*

Organisms isolated	Number of times isolated from soils[a]		
	Brookings, S.D.	Waseca, Minn.	St. Paul, Minn.
Fusarium sp.	4 p	3 p 1 s	1 p 3 s
Helminthosporium sativum	–	–	3 p
Alternaria sp.	–	–	1 p 1 s
Stilbaceae	–	1 p 1 s	–
Unknown fungi	1 p	3 s	2 p 3 s

[a] (p) indicates pathogenic, (s) indicates saprophytic.

Additional isolations were made from naturally infected soil obtained from the agronomy plots at University Farm, St. Paul, Minn., in which 1-year-old crested wheatgrass plants were severely damaged by root rot. For purposes of isolation, seeds of the Forage and Fairway strains of crested wheatgrass were planted in the greenhouse in the soil on which the diseased crested wheatgrass plants had been growing. Some of the root tissue of the resulting seedlings was surface-sterilized with mercuric chloride before being plated out, while some was washed and soaked repeatedly in sterile distilled water before being plated out on water agar and potato-dextrose agar. The following organisms were obtained: 14 isolates of *Pythium graminicolum*[2] Subramaniam, 8 isolates of *Fusarium* spp., 2 isolates of *Rhizoctonia solani* Kühn, 2 isolates of *Helminthosporium sativum* P.K. and B., 1 isolate of *Alternaria* sp., and 4 isolates of *Trichoderma* sp., along with 11 unknown fungi. Most of the cultures of *Pythium*[2] were obtained by the second method of isolation.

GREENHOUSE PATHOGENICITY TEST

The pathogenicity of some of the organisms obtained from the Waseca and St. Paul soils, as well as some from the agronomy plot soil, was tested in the greenhouse and the field. In the greenhouse *Helminthosporium sativum* isolated from the roots of *Bouteloua gracilis* grown in St. Paul soil, *Fusarium* sp. isolated from the roots of *Sorghastrum nutans* grown in Waseca soil, and *Rhizoctonia solani* and *Pythium graminicolum* isolated from the roots of crested wheatgrass grown in the agronomy plots at University Farm were introduced into the soil by distributing bits of mycelium on the surface of soil in 4-inch pots at the time of planting. This method of inoculation was used in all experiments except where otherwise stated.

[2] The cultures of *Pythium graminicolum* isolated from infected soil in the agronomy plots of University Farm, St. Paul, Minn., produce oogonia readily on cornmeal and potato-dextrose agars. When grown in potato-dextrose broth for 4 days and then transferred to sterile distilled water, the mycelium produces typically lobed sporangia, vesicles, and zoospores within 48 to 60 hours. The granular protoplasm passes from the sporangium into a vesicle at the end of a slender tube where it differentiates into 50 to 60 swarmspores per vesicle.

Three hundred seeds each of bromegrass and crested wheatgrass were planted in the above soils; and one planting was made in steamed soil as a check.

TABLE 2.—*Survival of Bromus inermis and Agropyron cristatum in the greenhouse in steamed soil inoculated with four fungi*

Organisms	Number of surviving plants of the two grasses[a]	
	A. cristatum	*B. inermis*
Helminthosporium sativum	0	29
Rhizoctonia solani	61	73
Fusarium sp.	66	96
Pythium graminicolum	0	38
None	72	116

[a] One hundred and fifty seeds of each species of grass.

It appears that *Helminthosporium sativum* and *Pythium graminicolum* reduced the stands of both grasses about equally. Preemergence killing was the most conspicuous type of injury caused by both. Of the brome seedlings that survived in the inoculated soil only a few died after emergence.

Stakman (6), Christensen (2), and Dosdall (4) reported about 20 years ago that *Helminthosporium sativum* could cause seedling blight and root rot of grasses. The writer's results confirm theirs. Sprague and Atkinson (5), and Buchholtz (1) have recently reported seedling blight and root rot of grasses caused by *Pythium*.

Field tests were next made. *Helminthosporium sativum, Rhizoctonia solani, Pythium graminicolum,* and *Fusarium* sp., isolated from crested wheatgrass grown at University Farm, St. Paul, Minn., were used to inoculate crested wheatgrass. The fungi were grown on 70 cc. of a mixture of 95 per cent sand and 5 per cent cornmeal by volume in 8-oz. jars. One hundred seeds of the Fairway strain of crested wheatgrass were mixed with each culture; and the seeds were then planted with the inoculated culture medium in 2-foot rows, 36 hours after mixing. Five 2-foot rows were planted in each of the 3 replicates, making a total of 1500 seeds planted for each treatment. Noninoculated steamed sand and cornmeal mixture was used for the control plots. The experiment was planted on September 9, 1941, and the stand count was made on October 25.

Although the experimental set-up was not such as to permit statistical analysis, *Helminthosporium sativum* reduced stands so consistently and so greatly that the results appear significant. *Pythium graminicolum* and *Fusarium* sp. reduced stands also, but very much less than *H. sativum*. The isolate of *Rhizoctonia solani* used did not cause appreciable damage; in fact, the average stand was somewhat better than that in the check plots, but because of variation in replicates it is doubtful if the difference is significant. Table 3 gives the stand counts for each of the replicates and the total stand for each treatment as a percentage of the check.

TABLE 3.—*Stand counts of the Fairway strain of Agropyron cristatum grown in the field in noninoculated soil and in soil inoculated with 4 fungi*

Organism	Stand count of replicates[a]			Total stand counts in per cent of check
	a	b	c	
Helminthosporium sativum	6	4	11	6.3
Rhizoctonia solani	149	150	132	129.4
Fusarium sp.	103	63	34	60.2
Pythium graminicolum	60	71	46	53.2
None	109	66	158	100.0

[a] Five hundred seeds sown in each replicate.

In further greenhouse experiments 14 species of grasses were tested against one isolate of *Pythium graminicolum* isolated from the roots of crested wheatgrass growing at University Farm, St. Paul, Minn. The tests were made in steamed soil that had been inoculated with the isolate at the time of planting. Check plants were grown in noninoculated steamed soil. Two stand counts were taken, 19 days and 46 days, respectively, after planting. Table 4 gives the stand counts of plants growing in the steamed soil and the inoculated soil and shows the amount of post-emergence killing. There was severe preemergence killing of all grasses and many seedlings were killed after emergence.

TABLE 4.—*Stand counts of 14 species of grasses grown in noninoculated steamed soil and in steamed soil inoculated with Pythium graminicolum in the greenhouse*[a]

Grass	Noninoculated soil		Inoculated soil	
	Number of plants surviving		Number of plants surviving after 19 days	Plants surviving after 46 days expressed in percentage of check
	After 19 days	After 46 days		
Agropyron cristatum	66	62	11	4.8
A. pauciflorum	46	40	28	42.5
A. smithii	45	50	16	32.0
Andropogon furcatus	21	15	3	6.8
A. scoparius	36	33	10	6.5
Bouteloua curtipendula	30	29	0	0.0
B. gracilis	31	27	0	0.0
Buchloë dactyloides	9	9	2	11.1
Elymus canadensis	14	14	1	7.1
E. junceus	48	46	7	8.7
Oryzopsis hymenoides	22	21	0	0.0
Panicum virgatum	62	44	3	0.0
Poa ampla	47	47	15	10.1
Stipa viridula	51	42	0	0.0

[a] Seventy-five seeds of each species planted.

EFFECT OF SOIL TEMPERATURE ON THE PATHOGENICITY OF PYTHIUM GRAMINICOLUM

It was considered possible that soil temperature might influence the pathogenicity of *Pythium graminicolum*. Therefore, seedlings of *Agropyron*

cristatum and *Bromus inermis* were grown at 7 soil temperatures in steamed soil and in steamed soil inoculated with *P. graminicolum*. The experiment was conducted in temperature tanks (3), the seeds being sown in 4-inch pots. In half of each pot 25 seeds of *B. inermis* were planted and in the other half 25 seeds of *A. cristatum*. Three isolates of *P. graminicolum* were tested, 2 pots of each isolate and 2 pots of noninoculated steamed soil being maintained at each soil temperature.

In this experiment temperature had no appreciable effect on the pathogenicity of any of the isolates (Table 5). Although *Bromus inermis* appeared slightly more resistant to isolate 59 than *Agropyron cristatum*, the stands of both grasses were markedly reduced by all 3 isolates.

TABLE 5.—*Seedling survival of Bromus inermis and Agropyron cristatum at 7 temperatures in soil inoculated with 3 isolates of Pythium graminicolum*

Soil temperature in degrees C.	Isolate and number of seedlings surviving 18 days after planting[a]							
	Bromus inermis				*Agropyron cristatum*			
	27	50	59	check	27	50	59	check
12.3	1	1	2	39	0	0	0	25
17.0	0	3	3	28	0	0	0	20
18.0	0	2	3	33	0	0	0	19
24.3	0	0	7	37	0	0	0	16
25.6	0	0	1	36	0	0	0	15
28.3	2	0	2	34	0	0	0	19
31.5	3	1	5	34	0	0	1	21

[a] Fifty seeds of each species planted in each treatment.

SUMMARY

Helminthosporium, Alternaria, Fusarium, Cladosporium, Penicillium spp., and many cultures of unknown fungi and bacteria were isolated from 1,500 seeds of 11 species of grasses.

Seed treatment with mercuric chloride did not produce significant differences in germination, emergence, or seedling vigor when the grasses were grown in steamed sand.

Preliminary pathogenicity tests on water agar, made with some fungi isolated from the roots of grasses grown in soils from Brookings, S. Dak., Waseca, Minn., and St. Paul, Minn., indicated that some organisms from each source were pathogenic.

Helminthosporium sativum, isolated from roots of *Bouteloua gracilis* grown in St. Paul soil and from the roots of *Agropyron cristatum* grown in soil obtained from the agronomy plots of University Farm at St. Paul, Minn., caused severe preemergence killing, reduction of root development, and stunting of *A. cristatum* and *Bromus inermis* in the greenhouse.

Pythium graminicolum, isolated from roots of *Agropyron cristatum* grown in soil obtained from the agronomy plots of University Farm, St. Paul, Minn., caused preemergence killing, reduction of root systems, and

stunting in all experiments. Post-emergence killing also was common in 4 of 14 species of grasses growing in soil inoculated with this organism.

The pathogenicity of *Pythium graminicolum* on *Bromus inermis* and *Agropyron cristatum* was similar at soil temperatures ranging from 12.3° to 31.5° C.

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THE EFFECT OF PHOTOPERIODISM ON THE DEVELOPMENT OF BUNT IN TWO SPRING WHEATS

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INTRODUCTION

In a recent abstract (4) reference was made to the bunt reaction of Hope and Marquis wheats when grown at different day lengths under greenhouse conditions. In these experiments, Hope plants, exposed to light (1) continuously, (2) 10–11 hours daily, and (3) 8 hours daily, developed 64.1, 17.5, and 0.8 per cent smut, respectively. The corresponding percentages for Marquis were 32.7, 17.8, and 1.9. These results have a bearing on studies concerning the nature of resistance. Further tests, therefore, were made to determine the effectiveness of continuous light in changing the bunt reaction of a variety known to be resistant under a wide range of field conditions.

METHODS AND EXPERIMENTAL RESULTS

Seed of the spring wheat variety Canus (C.I.[1] 11637) was inoculated with chlamydospores of *Tilletia levis* Kühn races L–1, L–2, and L–4 and of *T. tritici* (Bjerk.) Wint. races T–1, T–4, T–9, T–10, and T–12. Canus had been tested previously for its bunt reaction under a wide range of field conditions and found, when spring-sown, to be consistently resistant to all races used in this experiment (5). The inoculated seed was germinated and grown to a height of approximately 1 inch in soil at 10° C. and then transplanted to greenhouse benches at Arlington Experiment Farm, Arlington, Va. In 1940, one block of seedlings, containing duplicate rows of 72 plants per row, and in 1941 single rows with the same number of plants for each of the races listed in table 1, were subjected to continuous light, beginning 2 days after transplanting and continuing until noninfected plants were in the soft dough stage. Daylight was supplemented from 4 p.m. to 8 a.m. by 100-watt Mazda lamps suspended at a distance of approximately 24 inches above the plants. The light intensity at the top of the plants during this period was approximately 50 ft.-c. Electric fans were used to circulate the air around the artificially illuminated plants. This kept the night-air temperature within approximately + 3° C. of that in the control series. During the time of these tests, the period from sunrise to sunset averaged approximately 11 hours. A control series of Canus received no supplementary illumination. In 1941 comparable tests were made with the spring variety Ulka (C.I. 11478), which, under field conditions, is susceptible to all races used in this experiment, except T–10. In 1940, this variety was included only in the continuous light series.

It is clear from the results presented in table 1, that a high degree of resistance to each of the races was maintained when the plants were exposed

[1] C.I. refers to accession number of the Division of Cereal Crops and Diseases.

to an 11-hour light period under greenhouse conditions. However, exposure to continuous light effected a marked lowering of the resistance of Canus to certain races. The average percentages of heads smutted by races L–1, L–2, and L–4 in the 11-hour day series were 2, 4, and 3, respectively, while the corresponding percentages for the continuous light series were 42, 55, and 37. Similar results were obtained with *Tilletia tritici* race T–4. With this race an average of 4 per cent smut developed with the 11-hour day in contrast to 59 per cent in the series with continuous light. It is apparent also from the data in table 1 that the effect of continuous light in lowering the resistance of Canus is specific only for certain races. For example, with T–1, an average of 22 per cent bunt developed in the continuous-light series in contrast to the much higher percentages obtained with the races referred to above. Furthermore, the prolonged-light treatment was relatively ineffective in lowering the resistance of Canus to races T–9, T–10, and T–12 where average percentages of smut in the natural day and continuous light series were 1, 1, 0, and 4, 10, and 12, respectively.

In extensive field tests with Canus and Ulka, the latter variety was completely susceptible to all races except T–10 (5). With this exception, it was likewise susceptible in the present 11-hour day and continuous-light greenhouse experiments. It is noteworthy from the results in table 1, however, that the percentages of smut were slightly higher with all races under the latter conditions. Ulka, usually intermediate in its reaction to T–10 under field conditions, continued to be so in the 11-hour day greenhouse tests, but

TABLE 1.—*Reaction of Canus and Ulka to certain races of Tilletia levis and T. tritici when grown under two different day lengths in the greenhouse at Arlington, Va., in 1940 and 1941*

Variety	Race of smut	Percentage smut[a]				Average	
		1940		1941			
		11-hr. day (87)	24-hr. day (59, 58)	11-hr. day (83, 87)	24-hr. day (47, 47)	11-hr. day	24-hr. day
Canus	L– 1	3	39	0	45	2	42
	L– 2	6	36	1	74	4	55
	L– 4	6	28	0	46	3	37
	T– 1	1	16	1	27	1	22
	T– 4	8	54	0	63	4	59
	T– 9	1	3	0	5	1	4
	T–10	1	11	0	9	1	10
	T–12	0	12	0	12	0	12
Ulka	L– 1	.....	100	80	75	80	88
	L– 2	.....	98	69	73	69	86
	L– 4	.....	100	68	75	68	88
	T– 1	.....	92	71	79	71	86
	T– 4	.....	95	74	71	74	83
	T– 9	.....	75	64	83	64	90
	T–10	.....	25	16	56	16	41
	T–12	.....	98	68	77	68	88

[a] Figures in parentheses indicate number of days from emergence to heading for Canus (first) and Ulka (second).

the percentage infection was increased when the variety was grown under continuous light, the comparative infection percentages being 16 and 41, respectively.

In these experiments, differences were observed in the growth types of both infected and noninfected plants of Canus and Ulka in the two different light conditions. By comparison, the plants under continuous light were slightly less vigorous, produced fewer tillers, were approximately 3 inches shorter, and headed in a shorter period, than did those in the series without supplementary illumination. Data on the rapidity of growth, indicated by the relative number of days required for heading, are recorded in parentheses in table 1. Because of shortage of greenhouse space in 1940, Ulka was omitted in the control series, but in 1941, data were obtained on both Canus and Ulka. In 1941, Canus headed 36 days earlier under continuous light than in the 12-hour day and Ulka 40 days earlier. As noted in table 1, it was the plants grown under the 24-hour day that had the higher percentage of bunt. Thus it is evident that at least in these varieties certain races of bunt fungi, once established in the host, may keep pace with the growing point even when the culms are elongating at an exceptionally rapid rate.

The effect of illumination on the reaction of varieties to various smuts has been studied by other investigators. Reed (3) investigated its effect on the reaction of oats to *Ustilago avenae* (Pers.) Jens. and *U. levis* (Kell. and Sw.) Magn. Under artificial illumination, oats headed several days to 4 weeks in advance of the nonilluminated controls, but, contrary to some of the present results with wheat, oats gave no evidence of any change in either susceptibility or resistance of the varieties to the races used. Lasser (2) reported marked differences in the development of oat plants under various conditions of illumination, but no significant variation in the percentages of smut was recorded. Similar results were obtained in his tests with spring wheat varieties inoculated with *Tilletia tritici*. In preliminary tests, the writers have studied the effects of different day lengths on the development of *Ustilago tritici* (Pers.) Rost. in the soft red winter wheat variety Wabash (C.I. 11384). The number of days from seeding to heading was 81, 112, and 122 for continuous light, 11-hour-, and 8-hour-day periods, respectively. The percentages of smut infection for the corresponding periods were 67, 74, and 79, indicating a slight trend, but no very definite relation between rate of growth in the host and percentage of loose smut. It seems evident, therefore, that the effect of illumination in changing the host reaction to smuts is specific for certain hosts and races of the fungi involved.

DISCUSSION AND CONCLUSIONS

It is clear from the results obtained that continuous light has a marked effect in lowering the resistance of Canus wheat to certain races of *Tilletia levis* and *T. tritici*. By controlling the light factor, changes in the general vigor of the host plants were effected. It is questionable, however, if any of these changes in vigor can be correlated with the breakdown in smut re-

sistance. The reaction of Canus to the races used in the present test has previously been studied under a wide range of field conditions. When spring-planted, the range in vigor of growth at different stations was even greater than that observed under the natural day and under continuous light in the greenhouse; yet, in all of the spring-planted field tests, the variety maintained a high degree of resistance. There is evidence also that the resistance of Canus to some of the races used in the present test can be altered without appreciable change in the growth habit of the host. Canus was inoculated, among other races, with L–1, L–2, L–4, T–4, T–9, and T–10 and fall-planted at Pendleton, Oregon, in 1937 and 1938 (5). Although there was no appreciable difference in the vigor of growth of the host in the two seasons, it was resistant in 1937 to all races, and, in 1938, intermediate in reaction. It is noteworthy that in these same field tests, Canus was more susceptible to T–9 and T–10 than it was in the greenhouse under continuous light, indicating that some other factors were responsible for the change in reaction.

In field tests, low percentages of bunt frequently have been obtained in what appeared to be rapidly growing plants. It is often assumed that under these conditions the bunt fungus grew more slowly than the wheat plant and failed to keep pace with the growing point of the shoots. It is evident from the present results, however, that these races of the bunt fungi kept pace with the host under exceedingly rapid growing conditions. Possibly the rate of growth of the fungus is conditioned by certain nutritional changes in the host some of which are brought about by prolonged exposure of the host plants to light (1).

SUMMARY

Canus and Ulka wheats inoculated with 3 races of *Tilletia levis* and 5 of *T. tritici* were grown at natural-day and continuous-light conditions in the greenhouse at Arlington Farm, Virginia. Under natural-day conditions Canus was resistant to all races, while under continuous light a marked breakdown in resistance to certain races but not to others was observed. Ulka was completely susceptible to all but one race under both day-length conditions, and resistance to this one was lowered by the long-day treatment.

Differences in vigor of the host plants in the two light conditions were observed, but none of the changes could be definitely correlated with the breakdown in smut resistance.

The highest percentages of bunt were obtained in plants growing at an exceedingly rapid rate. It is suggested that the rate of growth of the bunt fungus may be conditioned by certain nutritional changes in the host that may have been brought about by prolonged exposure of the host plants to light.

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4. RODENHISER, H. A., and J. W. TAYLOR. Studies on environmental factors affecting infection and the development of bunt in wheat. (Abstract) Phytopath. **30**: 20. 1940.
5. ————, and C. S. HOLTON. Variability in reaction of wheat differential varieties to physiologic races of *Tilletia levis* and *T. tritici*. Phytopath. **32**: 158–165. 1942.

THE ROLE OF PACKING METHODS IN THE INCREASE OF ANTHRACNOSE OF HONEYDEW MELON FRUITS[1]

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(Accepted for publication July 14, 1942)

Anthracnose of cucurbits caused by *Colletotrichum lagenarium* (Pass.) Ell. and Hals. was responsible for a considerable loss in honeydew melon fruits (*Cucumis melo* L. var. *inodorus* Naud.) in the Crowley region of the Arkansas River Valley of Colorado during the growing seasons of 1939 and 1940. A serious problem from the standpoint of the shipper has been the increase of infection following packing. Large numbers of clean and apparently healthy melons became diseased, probably in transit, and, on arrival in eastern markets, frequently were either sold as culls because of anthracnose or rejected because of secondary rotting of the lesions by species of Fusarium.

In a preliminary study designed to determine whether apparently healthy melons were field-infected, 84 such melons were selected at random in fields in which anthracnose was present. These melons failed to develop any appreciable amount of anthracnose, even during incubation at high humidities and summer temperatures. Therefore, it was suspected that certain procedures used during the packing process might have been responsible for the spread of anthracnose. Shippers moved melons from the field by the truckload. A high degree of bruising and mechanical injury invariably resulted. Each truckload was then dumped into a common 2,000-gal. wash tank. The water in such a tank frequently was not replaced for days, and even weeks, at a time. From here the melons moved on rollers to brushes and under an overhead sprinkling system. They were then carried on a conveyor belt to the sorters, who first discarded the obviously diseased melons and then graded the balance.

THE ROLE OF WASH WATER AND INJURY IN FRUIT INFECTION

In order to study the possible role of wash water in initiating new infections of injured and uninjured melon fruits, a small-scale laboratory test was conducted. Twenty melons showing active anthracnose lesions were selected. Each diseased melon was gently turned for 5 minutes in 4 liters of cold tap water in a clean, glazed 4-gallon jar. Microscopic examinations and cultural tests of the water were made after washing 4, 8, 16, and 20 infected melons. In addition 3 clean melons, each with 3 small scalpel cuts and an artificially produced bruise were rinsed in the water at the time of each examination. Three melons with similar artificial injuries were rinsed in clean tap water as a control. All melons then were held under wet canvas

[1] Paper No. 151, Scientific Journal Series, Colorado Agricultural Experiment Station.

[2] Cooperative investigation between the sections of Botany and Plant Pathology and Pathology and Bacteriology.

at summer room temperatures for 14 days. The results of this study are shown in table 1.

TABLE 1.—*The role of infested wash water and injury in infection of honeydew melon fruits by C. lagenarium*

Infestation of wash water				Infection of healthy melons				
No. of infected melons washed	Total no. of anthracnose lesions on infected melons washed	Average no. of conidia per drop in wash water	Growth in culture plates	No. healthy melons rinsed	Total no. melons showing infection	Total no. of stabs and bruises	Total no. infections in injured areas	Total no. infections in uninjured areas
Control	0	0	−	3	0	12	0	0
4	7	0.5	+	3	3	12	9	0
8	22	2.0	+	3	2	12	5	0
16	53	3.0	+	3	2	12	4	1[a]
20	76	4.0	+	3	3	12	6	0

[a] Stem end infection. This appeared to be the result of field injury.

Table 1 indicates that as few as 4 infected melons were sufficient to infest the wash water with conidia of *Colletotrichum lagenarium*. In addition anthracnose developed only at the spots where the melons were injured. Further, where conidia were absent, as shown by the control series, no anthracnose infection developed even though the melons were injured.

THE TOXICITY OF CHLORINE TO THE CONIDIA OF COLLETOTRICHUM LAGENARIUM

Because of the known general disinfectant action of chlorine in water, its known fungicidal[3,4,5] and non-residual properties, and its relatively low cost, solutions containing varying concentrations of chlorine were prepared from calcium hypochlorite. Since 5 minutes was noted to be the minimum interval for washing melons at the packing and shipping stations, the first problem was to determine the lowest concentration of chlorine sufficient to inactivate the conidia of *C. lagenarium* within this time period. As a preliminary procedure spores of *C. lagenarium* were taken from pure cultures of the fungus and suspended in large quantity in a few milliliters of sterile water. A loopful of this spore suspension was then transferred to each of 6 test tubes containing, respectively, 3 ml. of a solution of 10, 40, 120, 300, 600, and 1,000 parts of chlorine per million parts of water. The concentration of chlorine was determined by titration with standard sodium thiosulphate solution. After 5-minute treatments the chlorine in each solution was inactivated by the addition of an amount of either 0.1N or 0.01N

[3] Ark, P. A., and J. T. Barrett. Phytophthora rot of asparagus in California. Phytopath. **28**: 754–756. 1938.

[4] Baker, Kenneth F., and F. D. Heald. Some problems concerning blue mold in relation to cleaning and packing of apples. Phytopath. **22**: 879–898. 1932.

[5] Tisdale, W. B., and S. O. Hawkins. Experiments for the control of phoma rot of tomatoes. Fla. Agr. Exp. Stat. Bull. 308. 1937.

sodium thiosulphate solution[6] slightly more than that necessary to react with the amount of chlorine originally present. Drops of these solutions were then streaked on plates of potato-dextrose agar. The results of this test are shown in table 2.

TABLE 2.—*The toxicity of varying concentrations of chlorine to the conidia of Colletotrichum lagenarium*

Concentration of chlorine (p.p.m.)	No. of conidia treated (per milliliter of suspension[a])	Growth of conidia in culture plates	Remarks
10	33,000	+	Light growth all plates
40	98,000	+	Five colonies on one plate only
120	188,000	−	No growth
300	138,000	−	No growth
600	82,000	−	No growth
1,000	58,000	−	No growth
Control + thiosulphate	83,000	+	Luxuriant growth all plates
Control	125,000	+	Luxuriant growth all plates

[a] Calculated from counts made with a ruled counting chamber.

Table 2 shows that concentrations greater than 40 parts of chlorine per million parts of water were sufficient to kill the conidia of *Colletotrichum lagenarium* suspended in water. The excellent growth of the control (untreated) spores and the spores treated with sodium thiosulphate indicated that the failure of conidia to develop in any culture plate was directly due to the chlorine treatment.

In a second study to determine the toxicity of varying concentrations of chlorine to conidia embedded in lesions on fruits, small pieces of melon tissue showing active anthracnose lesions on their surfaces were subjected to 5-minute treatments in solutions of varying concentrations of chlorine. After treatment each mass of tissue was carefully rinsed in sterile water. Following this a portion of the spore mass within a lesion was removed to a sterile water blank in order to obtain a spore suspension. Drops of this suspension were then streaked on plates of nutrient agar to determine whether the spores had been killed by the treatment. Solutions having concentrations of 40, 120, 300, 600, and 1,000 parts of chlorine per million parts of water were used in this study. It was found that a sufficient number of spores survived the treatments to give growth on the culture plates in all cases.

THE EFFECT OF CHLORINE TREATMENTS ON HONEYDEW MELONS

The effects of 5-minute treatments in chlorine solutions of varying concentrations were noted on several crates of firm, clean, ripe honeydew melons. Commercial preparations of both sodium and calcium hypochlorite were used in obtaining 2 series of chlorine solutions of 200, 1,000, 5,000, and 10,000 parts of chlorine per million parts of water. After treatment the

[6] American Public Health Association. Standard methods of water analysis. Eighth edition. N. Y. 1936.

melons were washed in running water, held at a temperature approximating that of an iced-refrigerator car (40° F.) for 7 days, and then at room temperature for another 14 days. At the end of each time period examinations showed that none of the treatments injured the melons.

SUMMARY

Preliminary studies regarding the increase of anthracnose of honeydew melons after harvest indicated that methods used by shippers were at fault. Washing both diseased and healthy melons in a common wash tank, coupled with rough handling of melons, appeared to be responsible for most of the increase in infection after packing.

Studies showed that as few as 4 diseased melons, when gently rinsed in 4 liters of water, were sufficient to provide a source of inoculum. When apparently healthy melons with artificially induced injuries and bruises were rinsed through this infested water, anthracnose developed only at the spots where the melons were injured.

Five-minute treatments in a solution containing 120 parts of chlorine per million parts of water proved adequate for the inactivation of the spores of *Colletotrichum lagenarium*. However, concentrations as high as 1,000 parts of chlorine per million parts of water failed to inactivate all of the conidia of *C. lagenarium* in active anthracnose lesions. Five-minute treatments in chlorine solutions with as high a concentration as 10,000 parts of chlorine per million parts of water did not injure honeydew melons.

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ANTHRACNOSE OF LUPINES[1]

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INTRODUCTION

In March, 1939, small plants of *Lupinus angustifolius* L. were found at Quincy, Florida, that were dwarfed and malformed, with some dying or dead. Enough plants were affected in certain areas to reduce the stand considerably. This field had been planted late as part of an experiment and the plants were only 4 to 6 inches tall at the time. No such condition was noted in earlier plantings and has not been seen since, although no special search has been made. The fact that no specimens of plants thus affected or no complaints have been received leads the writer to believe that the trouble is not serious. Nevertheless, the disease is potentially capable of causing much damage to seedlings and, since no previous record of the disease on this host is known, it seems worthwhile to record here the results of such observations and experiments as have been made.

SYMPTOMS

It was apparent that the plants in the field usually had been infected in the region of the cotyledons or through them. The cotyledons often were in the center of a lesion extending a considerable distance above and below them. In some cases the stem had been completely severed and the upper part or all of the plant killed. Other plants had a crook in the stem just above the lesion. Apparently, at one time the disease had partly girdled or greatly weakened the stem, so that it was unable to support the part above in its normal upright position. Some tops had turned at nearly a right angle to the main axis of the stem. Often there was still enough vitality in the stem to support growth so that the upper part resumed its original upright position, but left a crook (Fig. 1, A). The surface of the cankered area was brown and somewhat sunken. In some cases the surface of the lesion was slightly roughened by acervuli that had broken through the epidermis.

ETIOLOGY

Isolations were made from several of the plants collected in the field and a fungus was obtained in pure culture. When grown on oat agar this fungus produces a minimum of mycelium and large ochraceous salmon[2]-color spore masses. The spores are unicellular, hyaline, straight, often taper slightly at both ends, and measure 3.5–6.3 × 8.4–18.9 μ (average 4.6 × 13.3 μ), which is well within the range given by Shear and Wood[3] for conidia of *Glomerella*

[1] Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Georgia Agricultural Experiment Station, Experiment, Georgia. Paper No. 97, Journal Series, Georgia Agricultural Experiment Station.

[2] Ridgway, Robert. Color standards and color nomenclature. 43 pp., 53 color plates. (Washington.) 1912.

[3] Shear, C. L., and Anna K. Wood. Studies of fungus parasites belonging to the Genus Glomerella. U. S. Dept. Agr. Bur. Pl. Ind. Bull. 252: 1–110. 1913.

cingulata (Stonem.) Spauld. and v. Schrenk. A comparison of this fungus with *G. cingulata* from apple has failed to reveal any differences, either in morphology or the rate at which they decay apple fruits, which would seem to justify the belief that the lupine fungus is a distinct species. Hence it is assigned to *G. cingulata.*

Thus far the writer has been able to find only two other reports of anthracnose on lupines. Seymour[4] lists *Glomerella lupinicola* Dearness on an unidentified species of lupine. Dearness has informed the writer by letter that his species has never been published and that it got into Seymour's list through an oversight. A new species of *Gloesporium, G. lupinus,* has been described by Bonder[5] in São Paulo, Brazil. It is, however, not possible from his description to determine whether he had the same species as that reported in this paper.

INOCULATION EXPERIMENTS

Inoculation experiments, made several times, have shown beyond doubt that this fungus is capable of attacking lupine plants and causing symptoms similar to those seen in the field plants. In one experiment, set up on November 24, 1941, seedlings of *Lupinus angustifolius* were atomized with a suspension of spores in water and held in a moist chamber for 36 hours. Ten days after the inoculations were made the tips of some leaflets and petioles were dying and some infection had taken place through the cotyledons. Figure 1, B, was made on December 15, 1941. This shows a control plant at the left and two plants whose cotyledons were entirely destroyed and the stems severed. The leaf at the left has one typical lesion on the second leaflet from the right. This lesion resembles those illustrated on *L. albus* (Fig. 1, C). The disease did not make much headway in the greenhouse other than to continue to spread to a limited extent in the tissue already infected. When some of the plants were returned to the humidity chamber after a few days in the greenhouse, the disease made rapid progress. It appears from this and other experiments that, should dry weather follow an infection period, the progress of the fungus would soon be slowed down, and, if the plants had not been too severely damaged, they would resume growth and many would partially or wholly recover. During a prolonged wet spell, rainy or foggy weather sufficient to keep the plants wet, the fungus makes rapid headway and plants can be killed in a few days. Under such conditions the fungus fruits abundantly forming masses of slimy spores on the lesions, which supply an abundance of inoculum. These spores, especially while suspended in rain water, can be blown about by the wind, or carried by man or animals. Under conditions suitable for the fungus, infection takes place rapidly in the young leaflets, petioles, cotyledons, and stems.

In one experiment, spores from a culture were placed against the hypocotyl of young plants of *Lupinus angustifolius* just below the surface of the

[4] Seymour, A. B. Host index of the fungi of North America. (Cambridge, Mass.) Harvard University Press, p. 419. 1929.

[5] Bonder, G. Tremoco branco e suas molestias. Bol. Agr. São Paulo 13: 427–432. 1912.

soil to see whether infection could take place from the fungus in the soil. The plants were 10 days old and were growing in pots of sterilized soil in the greenhouse. The inoculations were made on October 29, 1941, and final notes taken on January 12, 1942. Only 2 plants out of 10 showed infection. These had large, dark-brown lesions on the hypocotyls, and many acervuli of the

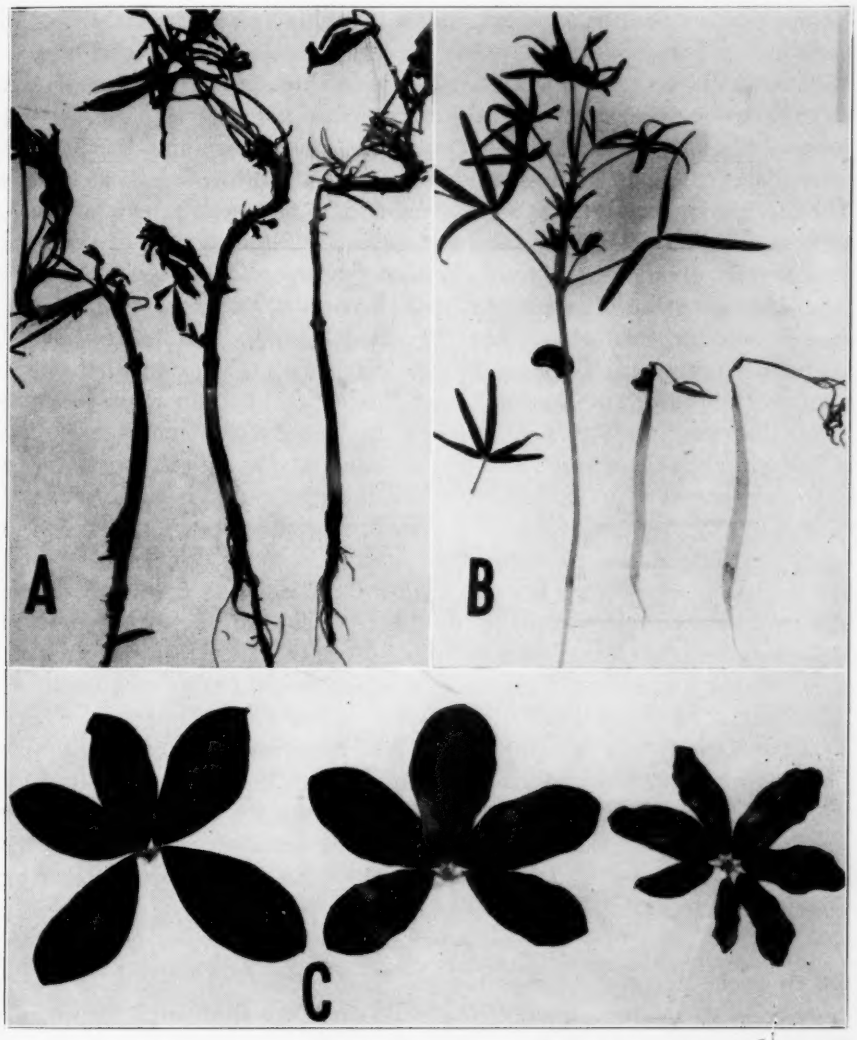

FIG. 1. Anthracnose of lupines caused by *Glomerella cingulata*. A. Three naturally infected plants of *Lupinus angustifolius* showing crooked stems and cankers at and above the cotyledons. The canker is especially conspicuous in the plant at the right. × ⅔. B. Two inoculated plants at the right in which the fungus had invaded and decayed the cotyledons and passed into the stems, severing them and causing the death of the parts of the plants above. At left a large control plant. The second leaflet from the right on the leaf at the left of the control has a lesion resembling those on *L. albus* leaves shown in C. × ⅔. C. Lesions on leaves of *L. albus* resulting from inoculation are shown on the two leaves at right. The lesions often coalesce, involving large areas of the leaflets. Control leaf at left. × ⅔.

fungus were present on their surfaces. Plants inoculated on the stems above ground failed to become infected. It seems that the stems are rather highly resistant to infection, except possibly below ground where seedlings may be infected if the soil moisture is rather high. Although this subject has not been thoroughly investigated, the experiments conducted suggest that, once the old cotyledons have fallen off and the leaflets matured fairly well, no serious damage need be expected, unless it be during an extremely long wet period.

That the leaves of *Lupinus albus* L. also can become infected was shown in an experiment in which a pot of seedlings was atomized with a heavy suspension of spores from a 4-day-old culture. The pot of plants was held in a screen house during a rainy period, and was covered by a cardboard carton. The inoculations were made on September 26, 1939, and 2 days later there were small, slightly sunken, water-soaked spots visible on most of the leaves. On the following day the tissue in these spots apparently was dead and turning gray. On the under sides of the leaves the lesions were considerably darker in color than above, but the exact details were obscured by the abundant pubescence. The spots were often up to 1 cm. in diameter, irregularly rounded, and scattered over the leaflet, but were most numerous along the margins (Fig. 1, C), where they were often confluent and had killed considerable areas. The upper sides of the lesions showed small brownish centers of undetermined origin, but assumed to be areas where the fungus hyphae were concentrated for the formation of acervuli. As the spots aged, the color darkened slightly, approaching straw color and having a narrow border of mummy brown (Ridgway) adjacent to the healthy tissue. The most severely injured plants were correspondingly retarded in growth. Unfolding terminal leaves were entirely killed. For the most part new buds were formed and growth eventually was resumed. Plants of *L. luteus* L. inoculated at the same time failed to show any infection.

These experiments indicate that anthracnose can be destructive both on *Lupinus angustifolius* and *L. albus* under high humidity and fairly high temperature conditions, especially in the seedling stage when leaves, petioles, cotyledons, and even the hypocotyls may be attacked directly and the stems be invaded by passage of the fungus into them from the adjacent more susceptible parts.

SUMMARY

An anthracnose of lupines not previously reported in this country is described. A comparison with *Glomerella cingulata* from apple showed that the fungus from lupine was morphologically the same and hence it was considered to be that species. The fungus attacks *Lupinus angustifolius* in the seedling stage under high humidity conditions. Young leaflets, petioles, stems, and cotyledons are susceptible. The underground portion of the hypocotyl may be attacked, but the main stem is seldom invaded, except at the apex or through the cotyledons or branches. Leaves of *L. albus* were infected and severely injured. In the single test made, *L. luteus* was not infected.

REACTION OF STRAINS AND VARIETIES OF BARLEY TO MANY PHYSIOLOGIC RACES OF STEM RUST[1,2]

F. R. Immer, J. J. Christensen, and W. Q. Loegering

(Accepted for publication September 22, 1942)

Reid[3] found a high correlation between the reaction of barley to a composite group of races of *Puccinia graminis tritici* Eriks. and Henn. in the adult plant stage and to race 56 in the seedling stage. Brookins[4] demonstrated that in crosses involving Peatland, reaction to races 19, 36, and 56 in the seedling stage was determined by the same factor pair that conditioned reaction to a large collection of races in the adult stage.

Since attempts are being made to develop a desirable agronomic variety of barley resistant to stem rust, it would be of considerable help in the breeding program if the reaction to stem rust, especially to many physiologic races, in seedling stages could be used as a criterion of resistance in the adult stage. Consequently, such a study was made with 20 physiologic races of stem rust.

MATERIALS AND METHODS

In this study were used two rust-resistant varieties, Peatland, C.I. 452, and Chevron, C.I. 1111; two susceptible varieties, Barbless, C.I. 5105, and Minnesota 462; and 20 hybrid strains. Nine of these strains came from a cross of Minnesota 462 × Peatland, and 11 from Barbless × Peatland. All these lines had proved to be resistant when grown in rod rows and tested against a composite of many races of stem rust in the field.

The 24 varieties and strains of barley were tested for seedling reaction to 19 races of *Puccinia graminis tritici* and one collection of *P. graminis secalis* Eriks. and Henn. in the greenhouse in 1940 and 1941. The following races of *P. graminis tritici* were used: 10, 14, 15, 17, 19, 21, 24, 29, 34, 40, 48, 49, 53, 55, 56, 59, 97, 139, and 147; these were furnished by the Division of Plant Disease Control of the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station.

Seed of each variety or strain was planted in 4-in. flowerpots and inoculated with a single race of rust. The reaction of each variety or strain to

[1] Published as Scientific Journal Series Paper No. 2014 of the Minnesota Agricultural Experiment Station.

[2] Cooperative investigations between the Minnesota Agricultural Experiment Station and the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

[3] Reid, D. A. A study of the inheritance of seedling and mature plant reaction to *Puccinia graminis tritici* in a cross of Wisconsin 38 × Peatland barley. M.S. thesis. University of Minnesota. 1938.

[4] Brookins, W. W. Linkage relations of the factors determining reactions to stem rust in barley. Ph.D. thesis. University of Minnesota. 1940.

each of the races of rust was recorded in the manner described by Stakman and Levine.[5]

EXPERIMENTAL RESULTS

The two varieties, Barbless and Minn. 462, susceptible to collections of many races of stem rust in the mature plant stage in the field, also were susceptible to all races in the seedling stage. The 22 varieties and strains of barley, resistant in the field, were resistant to 18 of the 19 races in the seedling stage. Seedlings of all varieties and strains of barley were susceptible to physiologic race 29. This particular collection of race 29 was obtained from aecia on barberry.

In order to determine the reaction of adult plants to race 29, rusted seedlings from a number of hybrid lines were allowed to grow and then were reinoculated at heading time in the spring of 1941. Although the rust reactions were obtained for only 6 hybrid strains in the adult stage, all the plants of these hybrids were again susceptible.

All varieties and strains of barley were resistant to the one collection of *Puccinia graminis secalis* to which they were tested.

These results indicate that the varieties of barley susceptible in the seedling stage to certain races of rust also are susceptible in the adult stage. The reaction in the adult stage and seedling stage was the same for all races tested, indicating that the reaction is of a physiologic nature.

SUMMARY

Two varieties of barley, normally susceptible to stem rust under field conditions, and two varieties and 20 hybrids, normally resistant in the field, were tested in the seedling stage for reaction to 19 physiologic races of *Puccinia graminis tritici* and one collection of *P. graminis secalis.*

The varieties and hybrids that were resistant in the field proved resistant to all physiologic races of *Puccinia graminis tritici,* except race 29, in the seedling stage, and were resistant also to the collection of *P. graminis secalis.* The two varieties susceptible in the field were susceptible to all races of *P. graminis tritici* in the seedling stage but resistant to the collection of *P. graminis secalis.*

All varieties and strains of barley tested were susceptible to race 29 in the seedling stage. Six of these strains were tested in the adult stage and found to be susceptible.

Seedling reaction to rust can be used to eliminate lines of barley which are susceptible in the adult stage.

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[5] Stakman, E. C., and M. N. Levine. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Minn. Agr. Exp. Stat. Bull. 8. 1922.

PHYTOPATHOLOGICAL NOTES

A Disease of Apple Grafts and Layers Caused by a Rhizoctonia.—A disease of underground portions of stems of apple nursery trees has been causing considerable loss in the experimental nursery at the Maryland Agricultural Experiment Station at College Park, Maryland. The methods used there for propagating apple trees on their own roots are: 1, Planting grafts in a furrow and drawing the soil to the young shoots as they grow, thus producing etiolated shoots that readily strike root; 2, pinning down in a furrow and covering with soil the tops of 2-year-old nursery trees and drawing the soil to the young shoots as they emerge from the buried tops, thus providing etiolated stems that readily form roots. Either the graft or the layer method of own-root propagation has the disadvantage, from the disease standpoint, of exposing young shoots that have the characteristics of herbaceous material to the attack of soil fungi of the damping-off type. In 1936, some losses occurred in the experimental apple nursery. In 1937 nearly all of a block of 10,000 grafted plants were killed, and in the layered plants there was a loss of about 10 per cent. In 1938, nearly 12,000 grafted plants were killed.

A similar disease has been causing some loss on apple seedlings (Fig. 1, C) at the U. S. Bureau of Plant Industry Station, Beltsville, Maryland. In certain places in the nursery, where conditions were favorable and the pathogen was present, a large percentage of the young seedlings were killed when 2 to 4 inches high.

The disease first becomes manifest as definite and distinct lesions on the young underground stem of a graft or layer. These lesions may advance very rapidly and soon girdle the stem, giving a damping-off effect (Fig. 1). In less severe cases the lesions may advance more slowly, and the shoot may grow to be a foot or more high before it is girdled and dies. Many times, especially when conditions are not favorable for infection, a large number of lesions occur on the underground stems; but they heal, and the next year the scars from healed lesions are very characteristic (Fig. 1, A, B). Another symptom usually present is the adherence of fungus filaments with enmeshed particles of soil to the leaves and stems (Fig. 1, A), in contact with the soil, thus producing a cobweb-like effect. This symptom is similar to that of a related disease on holly cuttings.[1]

Some apple varieties are much more susceptible than others; but more work must be done before attempting to list varieties in the order of their susceptibility or resistance. It was observed, however, that U.S.D.A. clone No. 227[2] showed fewer and smaller lesions the second year after inoculation of layer shoots than did any other of a number of clones or varieties.

When losses showed up in June, 1938, the writers began searching for the cause of the trouble. No reference to such a disturbance on grafts or layers

[1] Cooley, J. S. Defoliation of American holly cuttings by Rhizoctonia. Phytopath. 32: 905–909. 1942.

[2] This clone was introduced by Guy E. Yerkes for use as an understock.

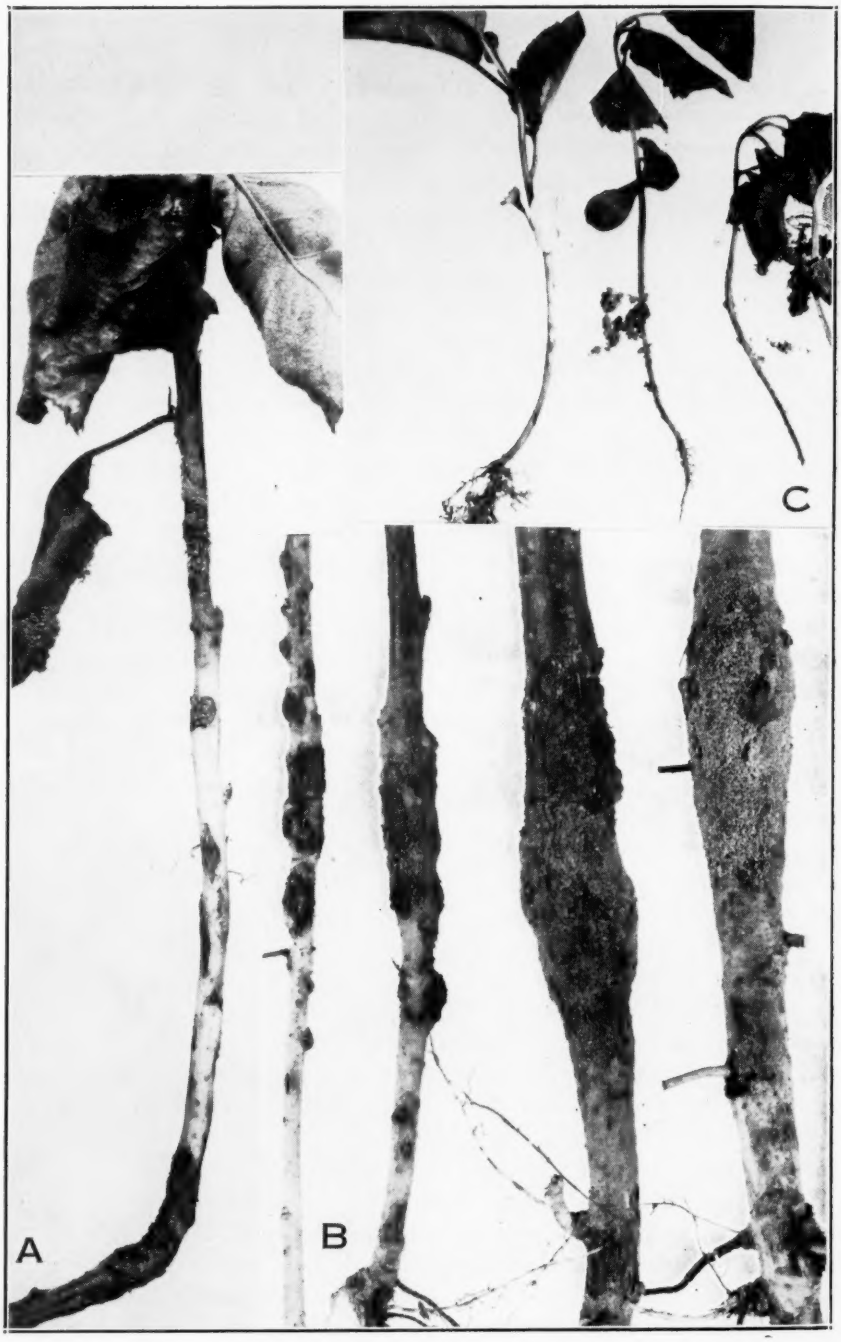

FIG. 1. Effect of Rhizoctonia on shoots of apple layers and on apple seedlings. A. Lesions, some causing girdling, on shoots of apple layers, also enmeshed grains of sand on the under side of affected leaves. B. Healing lesions and hypertrophy on layer shoots of apple trees the second summer after infection. C. Different stages in the killing of apple seedlings.

has been found in the literature. E. A. Siegler,[3] over a period of years since 1920, made observations and isolations from lesions on roots of apple and peach seedlings. He consistently isolated a species of Rhizoctonia from affected plants and has repeatedly observed sclerotia of Rhizoctonia on apple seedlings from commercial apple seedling nurseries. J. Perlberger[4] reported apple and other nursery trees of deciduous fruits affected with *Rhizoctonia bataticola* (Taub.) Butler group B. The disease, he says, sometimes destroys up to 60 per cent of the young trees in Palestine.

From the margin of dead and living tissue of active lesions of affected apple plants the writers have consistently isolated a species of *Rhizoctonia*.[5]

Flasks of sterilized oats inoculated with this fungus were used in the field as inoculum on young layer shoots. When this inoculum was placed underground around young layer shoots severe infection took place. Lesions were produced that were similar to those from which the inoculum was taken and a fungus apparently identical with the original inoculum was reisolated. In some cases where the conditions were favorable for infection, all the shoots in plots of 20 or more were completely killed, while the noninoculated checks in the rest of the nursery row showed no injury or only a trace.

An experiment looking to the control of this disease on grafts and seedlings in the nursery was carried out during the summer of 1940. Copper oxide dust in several concentrations diluted with talc, and also Bordeaux mixture and other fungicides were applied in the nursery on inoculated soil just prior to drawing the soil to the trees. The results of this work were inconclusive because conditions did not favor severe infection after the experiment was begun. The percentage of infections on the inoculated and untreated plots was so low that no conclusion could be drawn as to the effectiveness of any of the treatments.—J. S. COOLEY AND F. B. LINCOLN, Bureau of Plant Industry Station, Beltsville, Maryland and University of Maryland, College Park, Maryland.

A New Parasite of Tomatoes.—During the early fall of 1942, County Agent Kirk at Casper, Wyoming, sent in a few specimens of tomatoes with an infection, later identified as broom-rape, (*Orobanche ludoviciana* Nutt.).

The grower of these tomatoes, J. K. Kear, living near Casper, reported that this parasite was present on 50 plants out of 700 plants in one lot of the variety, 10-ton Rutger. Some of the infected plants were stunted while others were not and the tomato yield was light.

This was the first crop to be grown on the land since it had been reclaimed from native sod. No doubt the parasite had been active on sagebrush and possibly on other perennials where it is commonly found. How-

[3] Unpublished notes.

[4] Perlberger, J. *Rhizoctonia bataticola* (Taub.) Butler, in deciduous fruit-tree nurseries in Palestine. Palest, J. Bot. Hort. Sci. 1: 37–51. 1937. (Hebrew) abs. R. A. M. Vol. 16, Part 10, October, 1937, pp. 683, 684 Kew.

[5] A culture of this fungus was sent to Ernest Wright, who is making a study of forest-tree seedling diseases. He gave the opinion that the fungus is *Rhizoctonia solani*.

ever, it is of significance that *Orobanche* was found on an annual plant rather than on a perennial one, and it is thought to be novel in that respect.

FIG. 1. Tomato plant with its broom-rape parasite.

The accompanying cut shows the relative size of the parasite and its point of attachment.—G. H. STARR, Wyoming Agricultural Experiment Station, Laramie, Wyoming.

Two Items of Pathological History from California.—Evidence has been found in California agricultural literature to indicate considerably earlier dates for certain events of importance in plant pathology, than those ascribed to them in a recently published text book.[1] One of these relates to the use of copper as a fungicide.

"The use of copper salts as fungicides was first made popular in 1873, when copper sulphate solution was used to disinfect wheat seed carrying the bunt organism."

(Melhus and Kent, p. 99). Early volumes of *Trans. Cal. State Agr. Soc.* contain many references to treatment of seed wheat with bluestone to prevent

[1] Melhus, Irving E., and George C. Kent. Elements of Plant Pathology. Macmillan Company (N. Y.). 493 pages. 1939.

smut, as, for instance, the article in volume 1 (1858), which includes (p. 343) the following statement from M. Walthall, Jr. of Stockton.

"Seed wheat I wash in clear water, if there be smut in it, and soak it in very strong blue stone water—say one pound to eight gallons—about twenty minutes, and then roll it in fresh slaked lime, and sack it for use."

The short-lived California *Culturist* (1858–1861) also contains many references to the copper sulphate treatment, including (Vol. 1, p. 350, 1858) an account of an experiment in which the efficacy of the method was convincingly demonstrated. In an address at the State Fair of 1860, John Bidwell of Chico, one of California's most reliable early observers, stated (*Transactions*, 1860, p. 325) that he had been familiar with grain growing in the Sacramento Valley since 1841, and that smut (bunt) first appeared there suddenly in 1854 in a field sown with Australian seed. He endorsed the bluestone treatment, which, by 1860, was apparently almost universal with California wheat farmers. In 1867 a San Francisco firm made the statement (*Transactions*, 1867, p. 217)

"During the years 1853, 1854, 1855 and 1856 our grain was badly injured by smut, which the use of blue vitriol has remedied."

Regarding peach leaf curl, Melhus and Kent state, page 298,

"The pathogen, (*Taphrina deformans*) probably was introduced into the United States in the early Eighties of the last century."

No reference to the source of this information is given, but one wonders if it may have originated in the statement by Owens,[2] p. 262,

"The writer has been unable to find any mention of this disease in the American literature prior to 1883."

Many early American books on fruit growing, like Downing's "Fruits and Fruit Trees of America" (1845), describe peach leaf curl. In California Harkness[3] states, "I first observed this in the State in 1855." From 1858 on, the disease was a very common subject of discussion in the horticultural press of California, the natural inference being that the pathogen had been introduced into the State on some of the first peach nursery trees, which were brought in about 1850, and frequently thereafter. The *Transactions* for 1860 (p. 283) contained an essay on the subject of curl leaf which concludes,

"It is caused by the sudden change of the atmosphere from heat to cold while the foliage is in young and tender state of growth. As curl is caused by the action of the atmosphere, and that being governed by the Creator, I believe it to be a matter of impossibility for man to prevent the ravages of curl, to any great extent."

Harkness,[4] in a later article, made the dubious suggestion that *P. deformans* is identical with an indigenous fungus now called *T. Aesculi* (E. and E.)

[2] Owens, Chas. E. Principles of Plant Pathology. John Wiley and Sons, Inc. (N. Y.). 629 pages. 1928.

[3] Harkness, H. W. Fungi injurious to fruit trees. Rpt. California State Bd. Hort. Commsrs. **1883:** 51–59. 1883.

[4] Harkness, H. W. The curled leaf. Zoe. **1:** 87–88. 1890.

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Giesenhagen, on California buckeye. A different light on the history of peach leaf curl in California is thrown by a statement by John T. Strentzel,[5] one of the State's most respected early horticulturists, who arrived in 1850 and took a special and very intelligent interest in fungus diseases.

"In California, previous to the year 1852, a few peach trees were found about the Missions, around Los Angeles, the fruit of small size, white or yellow, globular, with a deep suture, *the trees badly affected with curl leaf.*" (The italic is ours). "Later, when budded trees, imported trees, began to be plenty, their vigorous growth and luxuriant foliage were characteristics distinguishing them from the so-called Mission peach. A few years later the curl began to appear also on budded trees."

It would be interesting to know the history of this disease in Mexico, occupied by Spanish settlers for 250 years before colonizing Alta California. It is commonly assumed that their fruit trees (except the fig, olive and vine) were all seedlings, but it may not have been impossible for them to have transported living trees on occasion. C. W. Reed of Yolo County (*Transactions*, 1867, p. 231) in 1856, "imported about eighty thousand trees, mostly grafted," and that was more than ten years before the opening of the transcontinental railroad.—RALPH E. SMITH, Division of Plant Pathology, University of California, Berkeley.

[5] Strentzel, John T. The peach tree and its diseases. Pacific Rural Press **20**: 182. 1880.